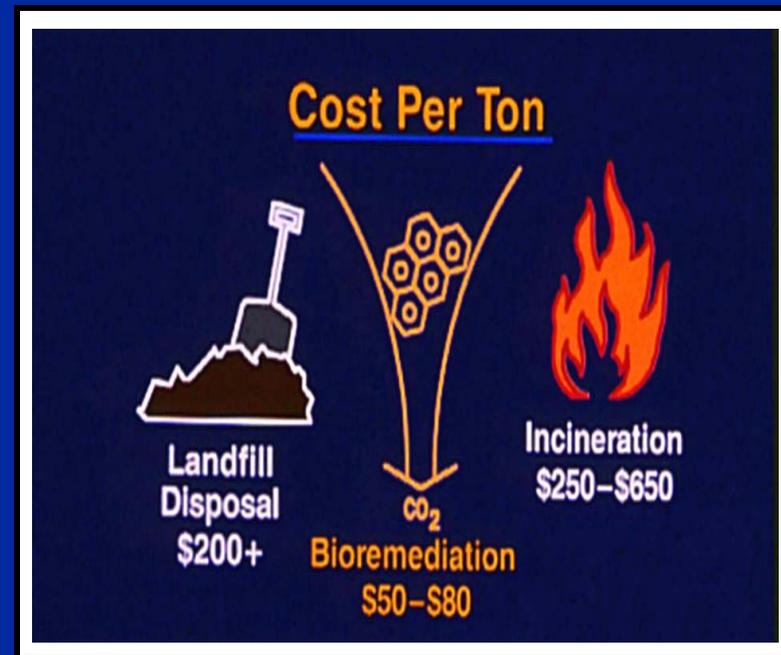


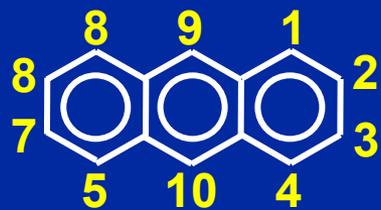
**High Molecular Weight Polycyclic Aromatic  
Hydrocarbon Degradation by *Mycobacterium*  
Species: Metabolism, Proteomic and  
Genomic Approaches in the Elucidation of  
PAH Biodegradative Pathways and  
Implications in Bioavailability**

**Director, Division of Microbiology  
National Center for Toxicological Research  
US Food and Drug Administration  
Jefferson, Arkansas 72079  
Carl E. Cerniglia, Ph.D.**

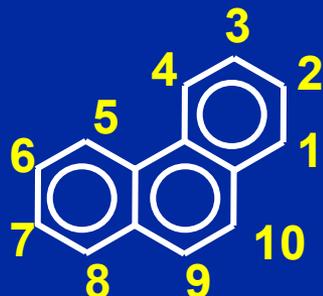
# Outline

- Significance of PAHs as Environmental Carcinogens
- Toxicology/Risk Assessment
- Overview of Microbial Degradation of PAHs
- *Mycobacterium* sp. PYR-1 Story
- Proteomic and Genomic Approaches
- Conclusions

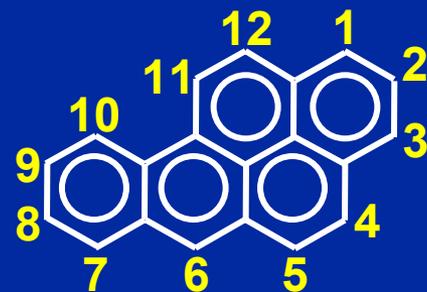




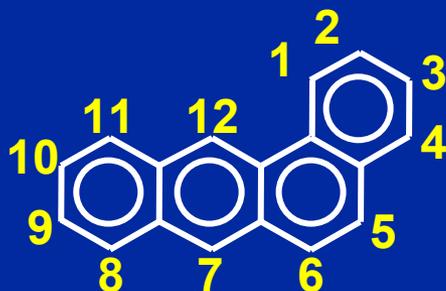
**Anthracene**



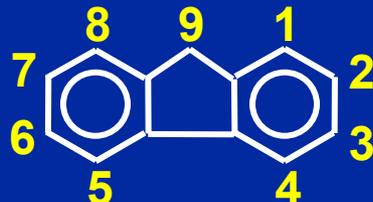
**Phenanthrene**



**Benzo[a]pyrene**



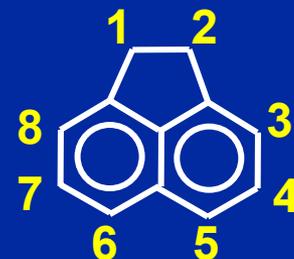
**Benz[a]anthracene**



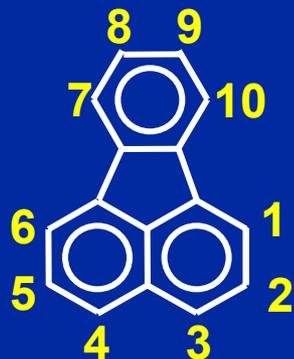
**Fluorene**



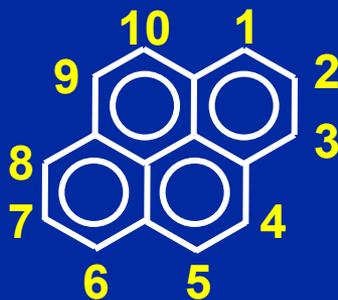
**Chrysene**



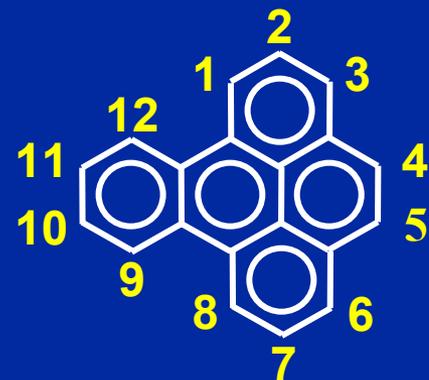
**Acenaphthene**



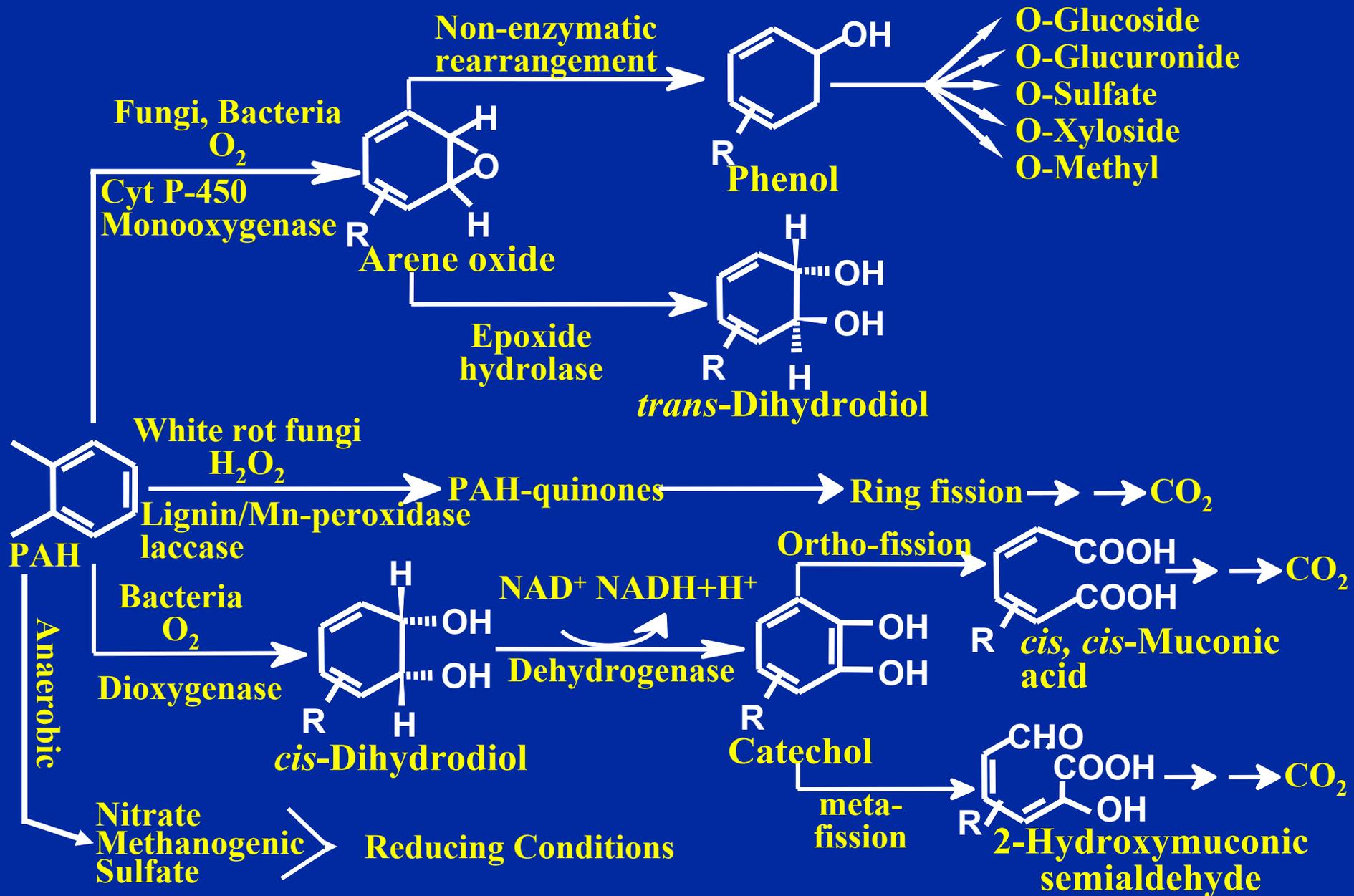
**Fluoranthene**



**Pyrene**

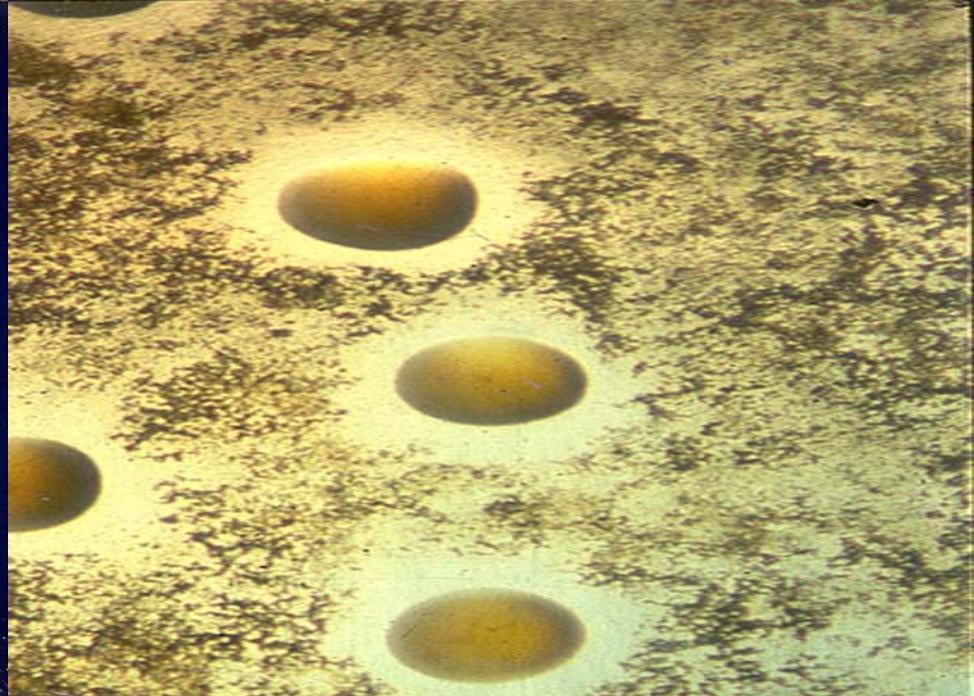


**Benzo[e]pyrene**

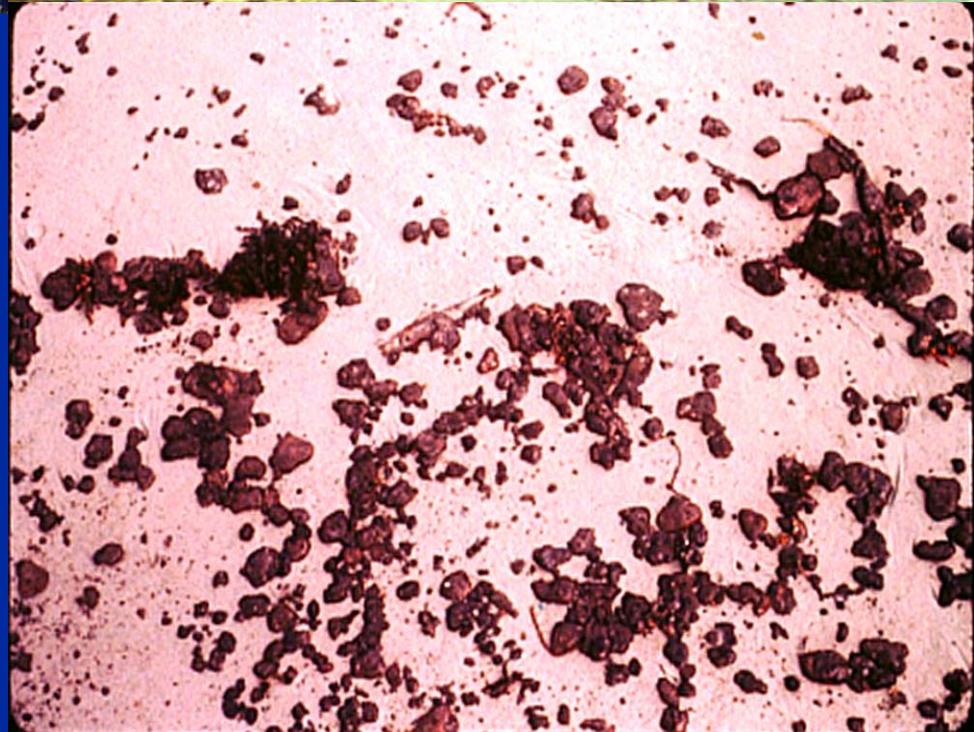


# Bacteria Oxidizing PAHs

BACTERIA	NAPH	PHEN	ANTH	PYR	FLU	BA	B[a]P
Acinetobacter	X	X					
Acromobacter	X						
Aeromonas	X	X					
Alcaligenes	X	X	X	X	X		
Arthrobacter		X			X		
Bacillus	X						
Brevibacterium		X					
Burkholderia	X	X					
Comamonas		X	X				
Corynebacterium	X						
Cycloclasticis	X	X	X				
Flavobacterium		X	X				
Micrococcus		X					
Moraxella	X						
<b>Mycobacterium</b>	<b>X</b>						
Nocardia		X	X				
Pasteurella					X		
<b>Pseudomonas</b>	<b>X</b>						
Rhodanobacter							X
Rhodococcus	X	X	X		X		
<b>Sphingomonas</b>	<b>X</b>	<b>X</b>	<b>X</b>			<b>X</b>	<b>X</b>
Stenotrophomonas		X		X		X	X
Streptomyces	X	X		X	X		
Vibrio		X					

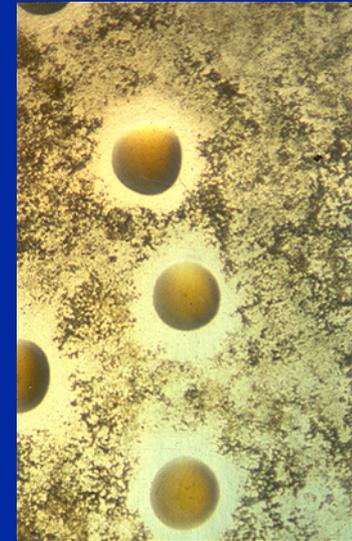


***Mycobacterium* sp. PYR-1**



# *Mycobacterium* sp. PYR-1

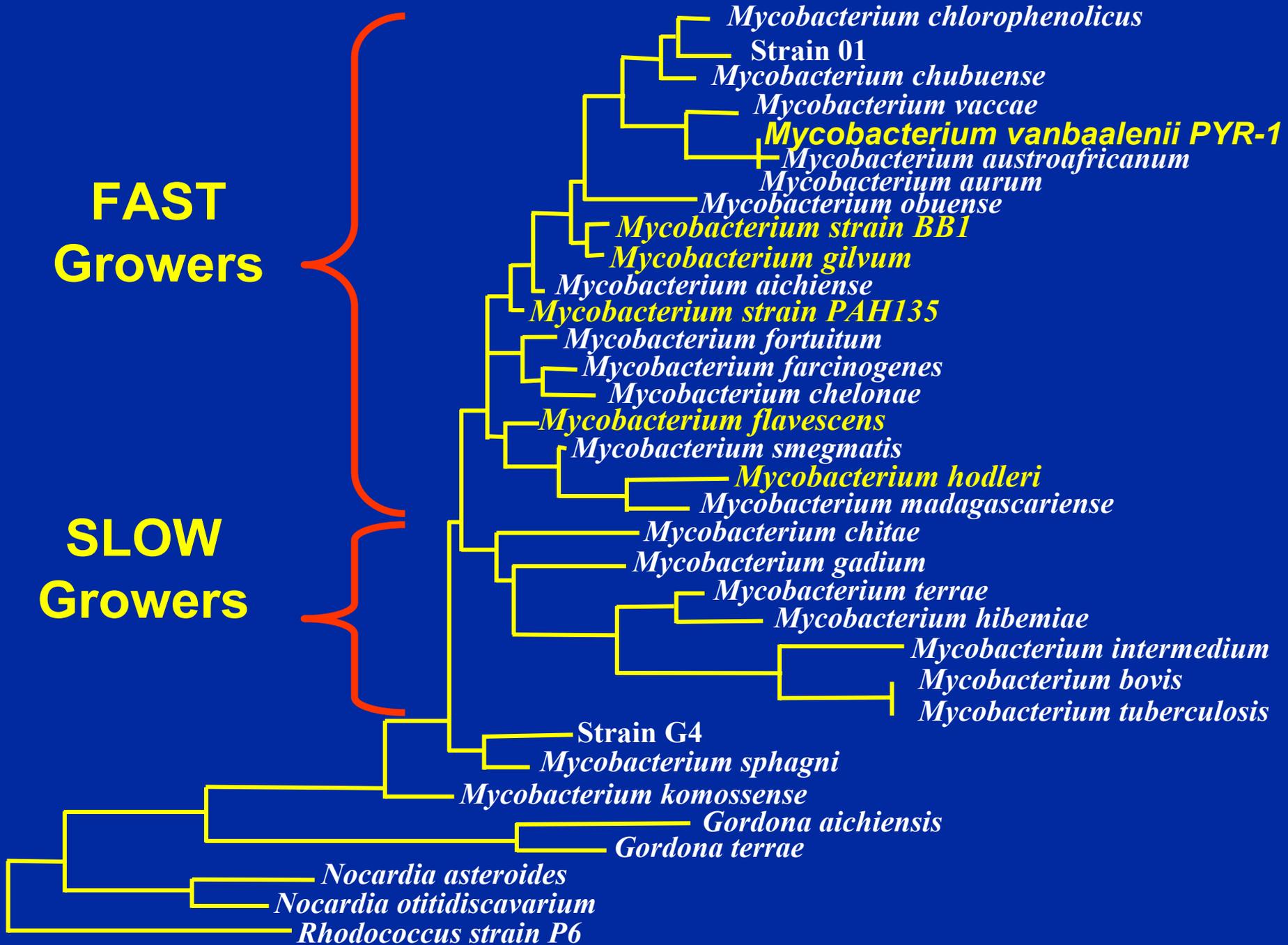
- Isolated from oil contaminated marine sediment
- Yellow pigmented colonies
- Acid-alcohol fast
- G + C content = 66.7%
- C<sub>60</sub>H<sub>120</sub>O<sub>3</sub> and C<sub>62</sub>H<sub>124</sub>O<sub>3</sub> mycolic acids
- Biochemical tests
- Fatty Acid Methyl Ester (FAME) profiles
- SDS-PAGE-soluble cellular proteins
- Dot-blot DNA hybridization
- PFGE analysis of restriction digested total genomic DNA
- 16S rDNA phylogenetic analysis
- *Mycobacterium vanbaalenii* PYR-1



Heitkamp, M. and Cerniglia, C. (1988) Appl. Environ. Microbiol. 54, 1612-1614.  
Rafii, F., Butler, W., and Cerniglia, C. (1992) Arch. Microbiol. 157, 512-520.  
Wang, R., Cao, W., and Cerniglia, C. (1995) FEMS Microbiol. Lett. 130, 75-80.  
Khan, A.A., Kim, S.-J., Paine, D.D., and Cerniglia, C.E. (2002) Int. J. Syst. Evol. Microbiol. 52, 1997-2002.

**FAST  
Growers**

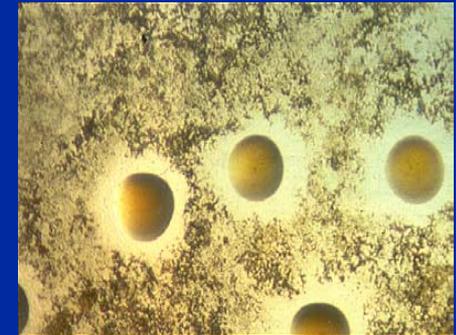
**SLOW  
Growers**



# Dr. Chase Van Baalen 1925-1986



- Professor of Botany and Marine Studies and Research Scientist (1973-86) at the University of Texas at Austin, Marine Science Institute located at Port Aransas, Texas.
- Internationally known alga physiologist, he had a remarkable devotion for experimental science.
- For his inspiration and assistance in determining the best sites to isolate PAH degraders from contaminated marine sediments along the Texas Gulf coast.



*Mycobacterium  
vanbaalenii* PYR-1

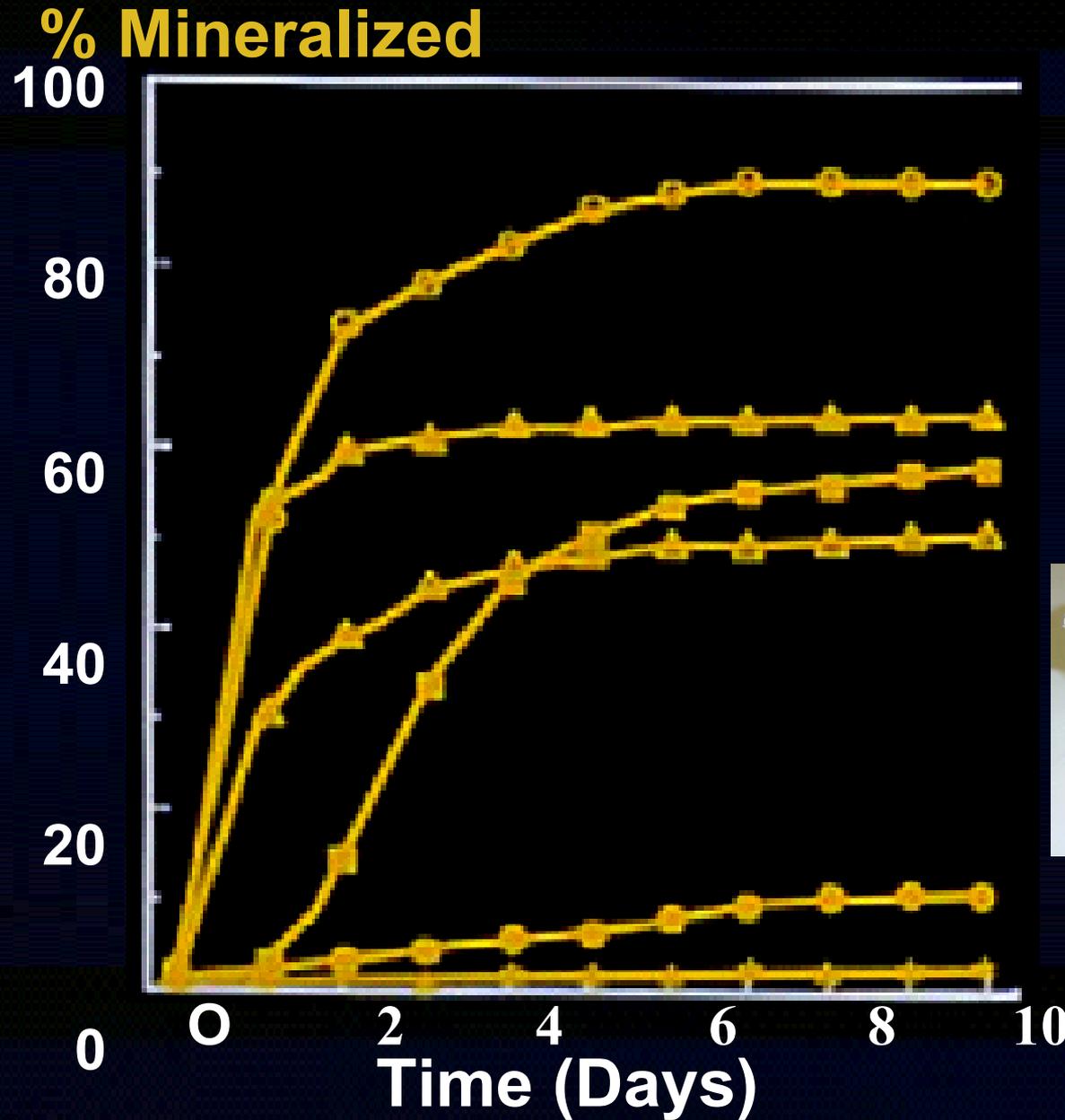
## Hydrophobicity of *Mycobacterium austroafricanum* GTI-23, in comparison with various other PAH-degrading isolates

Isolate	Medium	Culture age	Hydrophobicity (%)
<i>Acidovorax temperans</i> GTI-19	MSM/phenanthrene	1 day	3
<i>Burkholderia</i> sp. GTI-3	MSM/phenanthrene	6 days	15
<i>Pseudomonas viridiflava</i> GTI-5	MSM/phenanthrene	6 days	4
<i>Sphingomonas</i> sp. GTI-7	MSM/phenanthrene	2 days	15
<i>Sphingomonas</i> sp. GTI-8	MSM/phenanthrene	6 days	26
<i>Sphingomonas</i> sp. GTI-10	MSM/phenanthrene	6 days	0
<i>Sphingomonas</i> sp. GTI-11	MSM/phenanthrene	6 days	18
<i>Sphingomonas subarctica</i> GTI-12	MSM/phenanthrene	2 days	0
<b><i>Myco. austroafricanum</i> GTI-23</b>	<b>YPS/phenanthrene</b>	<b>6 days</b>	<b>48</b>
<i>Myco. austroafricanum</i> ATCC 33464	YPS	6 days	41
<b><i>Mycobacterium</i> sp. PYR-1</b>	<b>YPS/phenanthrene</b>	<b>6 days</b>	<b>52</b>

Bogan et al., (2003) Journal of Applied Microbiology 94:230-239.

# Research Goals

- To determine if *Mycobacterium* sp. PYR-1 has the ability to metabolize PAHs.
- To determine the metabolites produced by *Mycobacterium* sp. PYR-1 and elucidate the metabolic pathways.
- To determine biological/toxicological activity of the metabolites.
- Characterization of enzymes involved in the metabolism of PAHs.
- Elucidation of the enzymatic mechanisms and genes for enzymes that mediate PAH metabolism.
- Determine the potential of *Mycobacterium* sp. PYR-1 for *in situ* biodegradation of PAHs.



**Fluoranthene**

**Pyrene**

**Naphthalene**

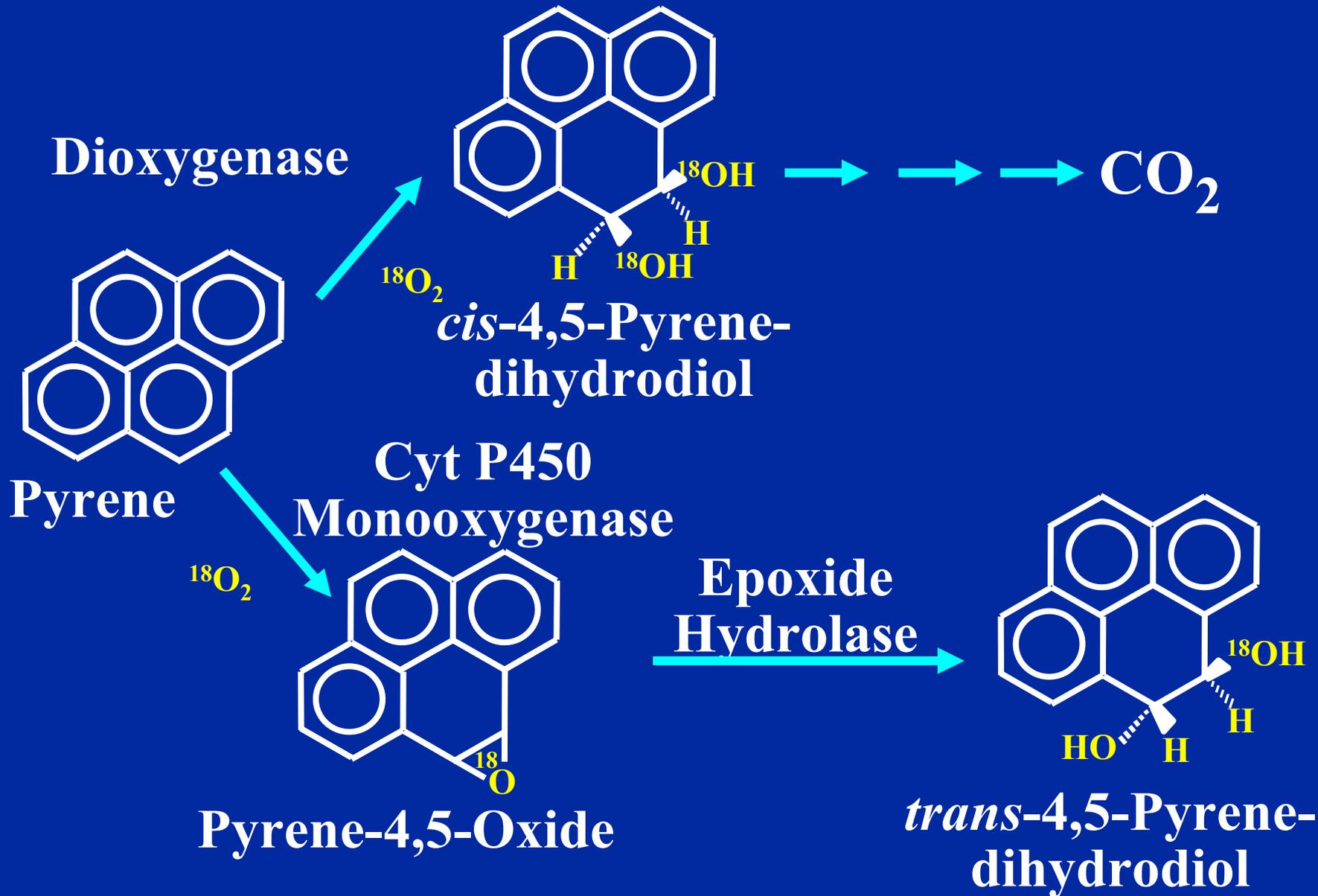
**Phenanthrene**



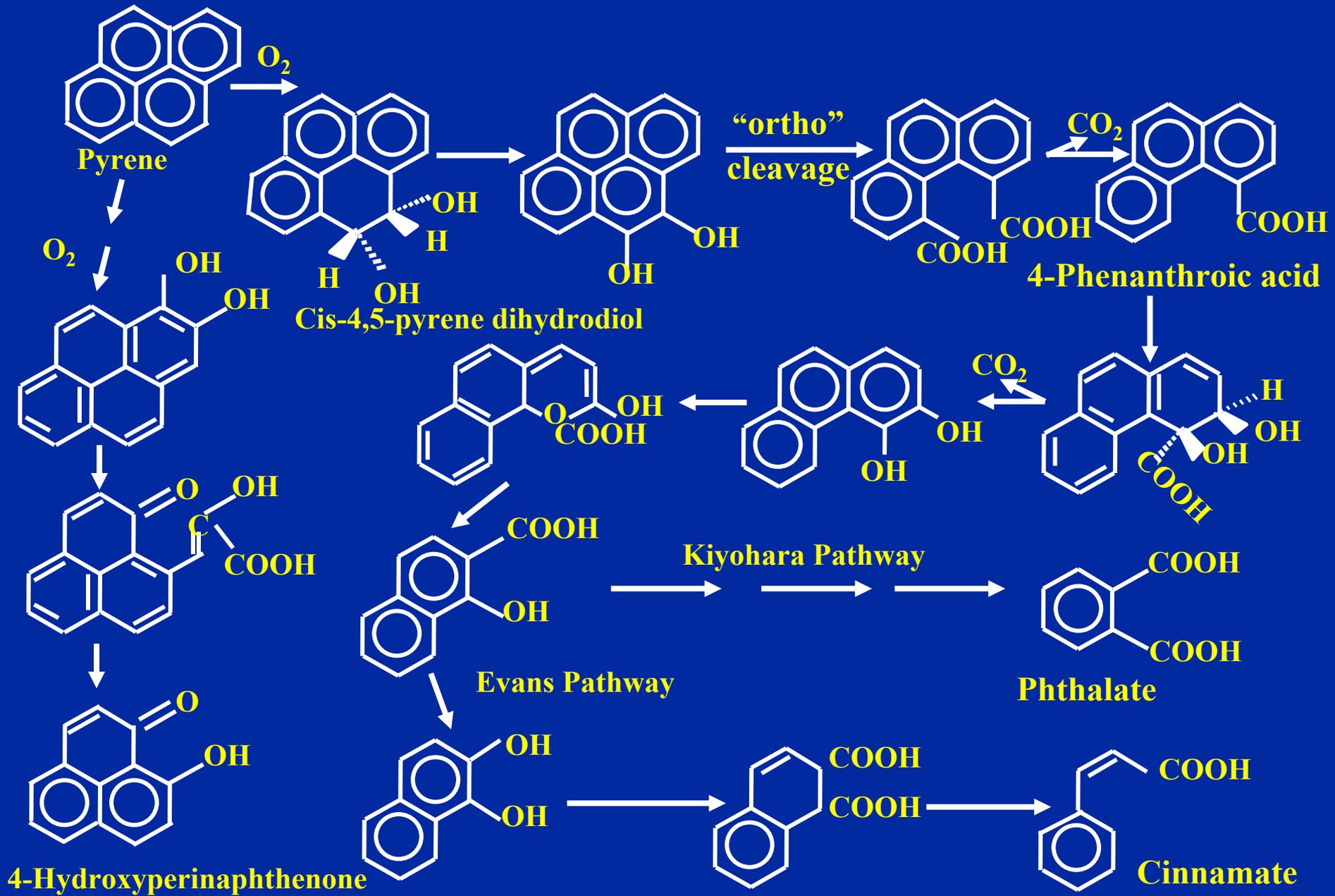
**1-Nitropyrene**

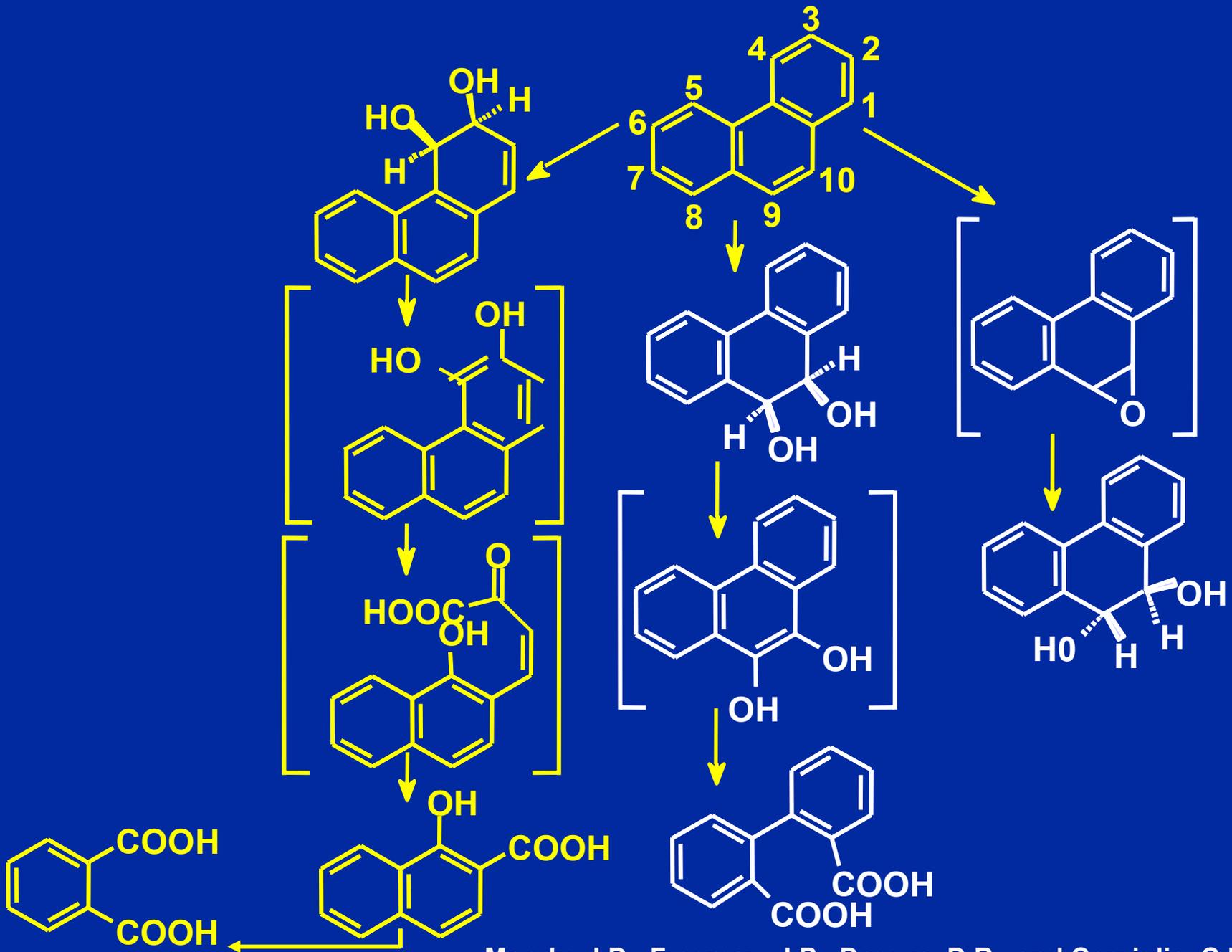
**6-Nitrochrysene**

**3-Methylcholanthrene**

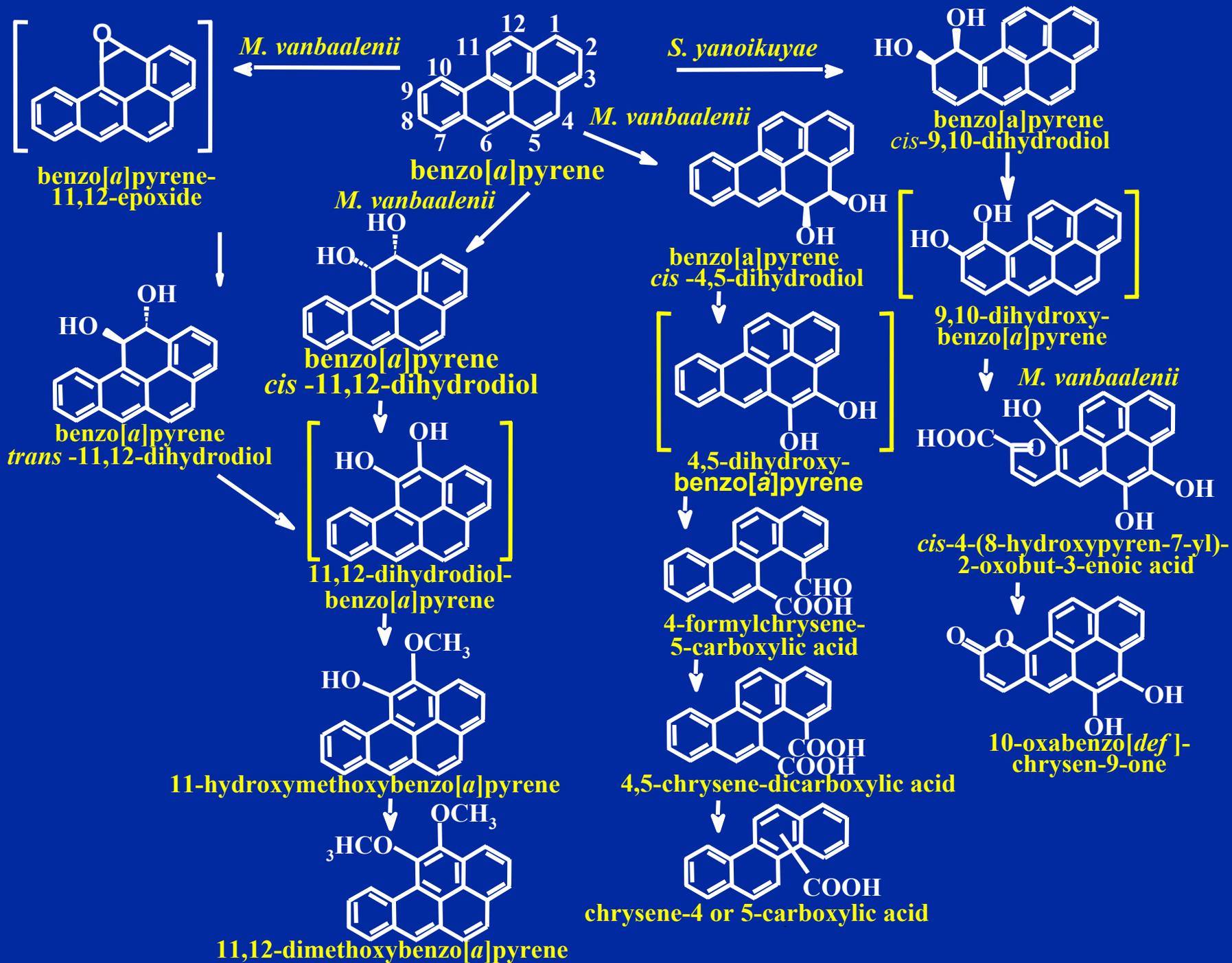


Heitkamp, M.A., Freeman, J.P., Miller, D.W., and Cerniglia, C.E. (1988) Appl. Environ. Microbiol. 54, 2556-2565.

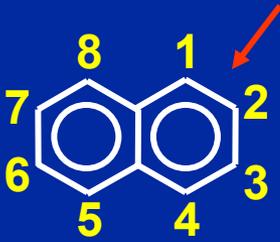




Moody, J.D., Freeman, J.P., Doerge, D.R., and Cerniglia, C.E. (2001) *Appl. Environ. Microbiol.* 67:1476-1483.



# Initial Metabolism of PAHs by *Mycobacterium* sp.



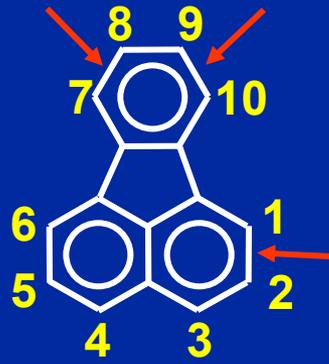
**Naphthalene**



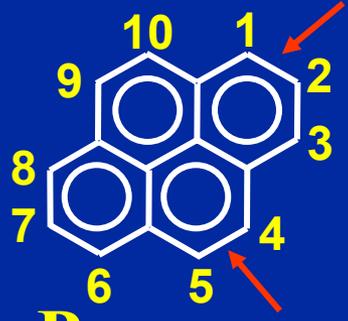
**Anthracene**



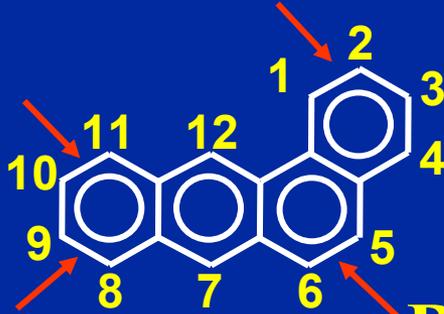
**Phenanthrene**



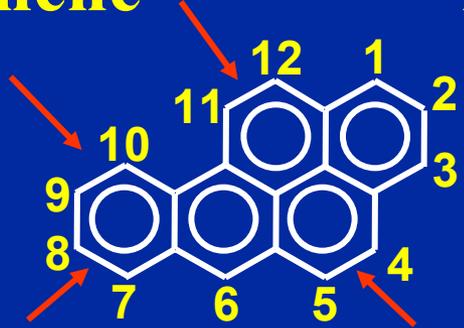
**Fluoranthene**



**Pyrene**



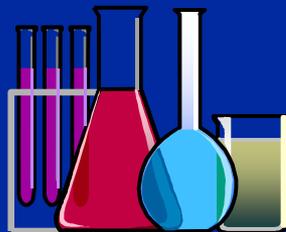
**Benz[a]anthracene**

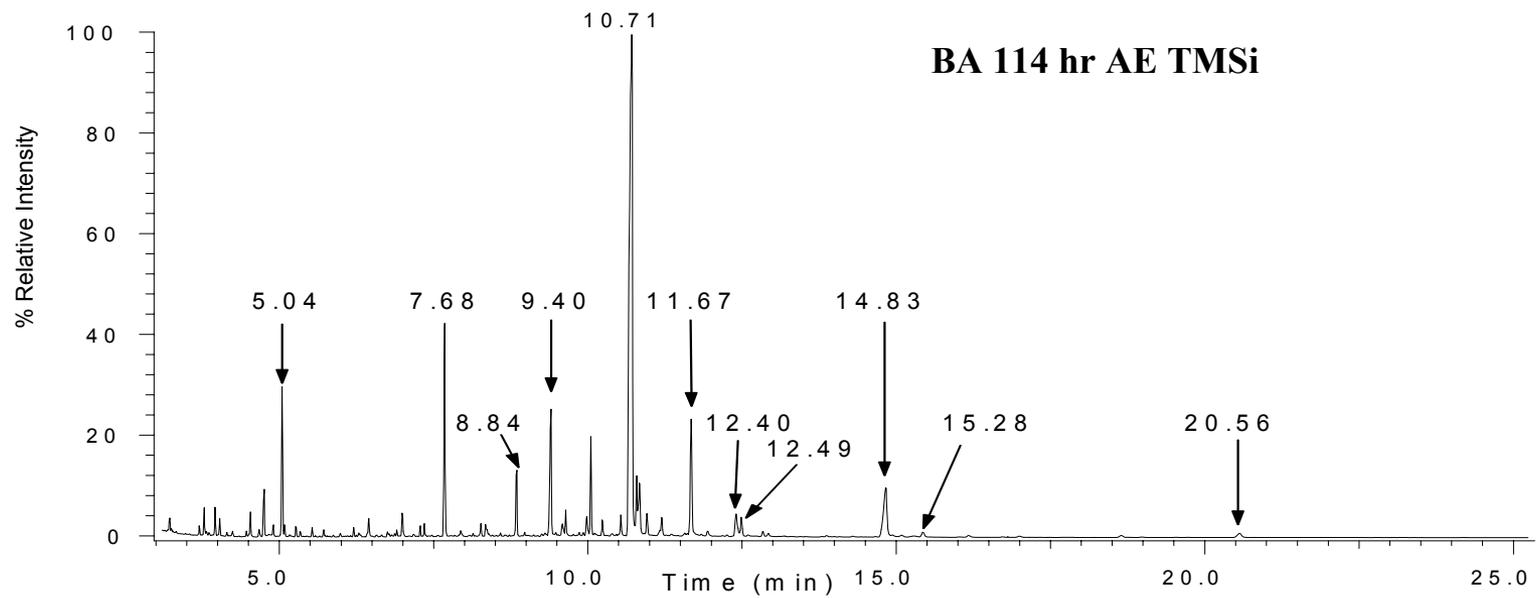
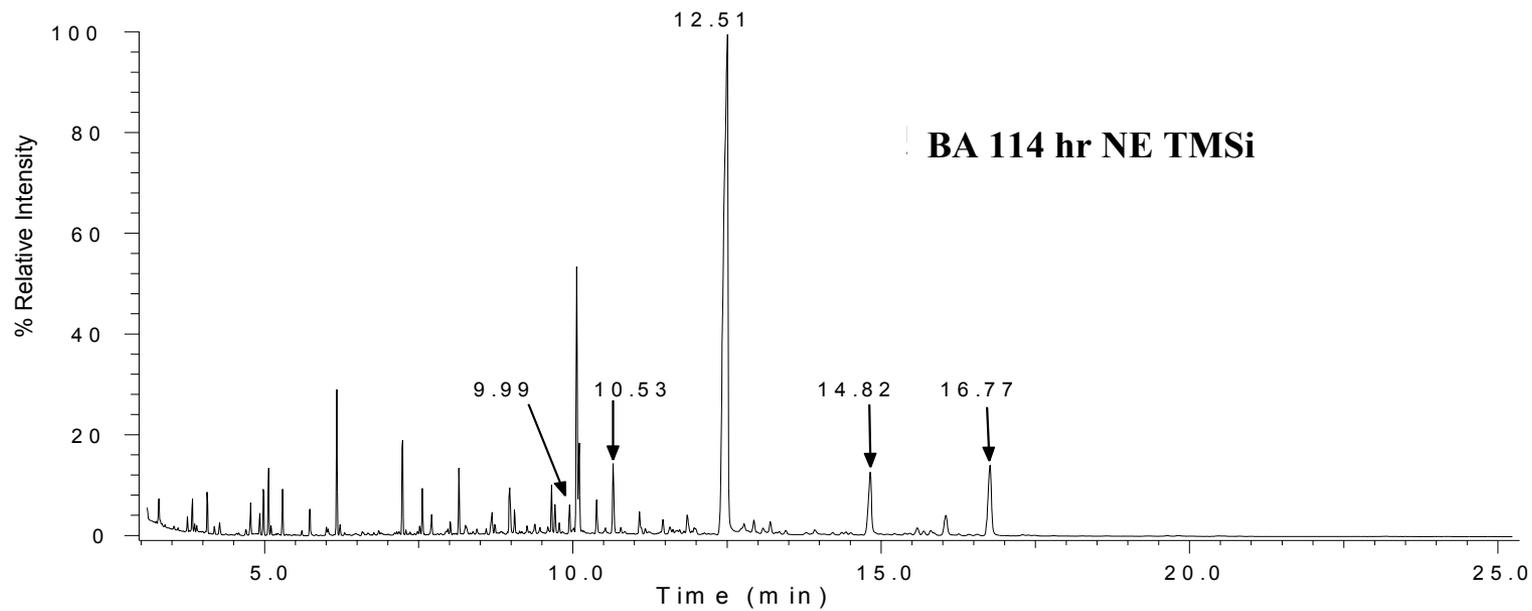


**Benzo[a]pyrene**

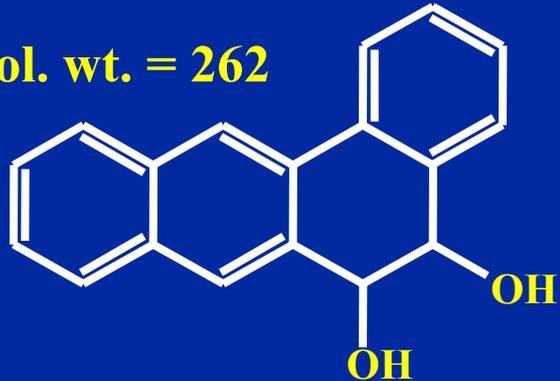
# Benz[a]anthracene BA-5,6-Dihydrodiol Metabolism

Trimethylsilyl  
Derivatization  
& GC/MS





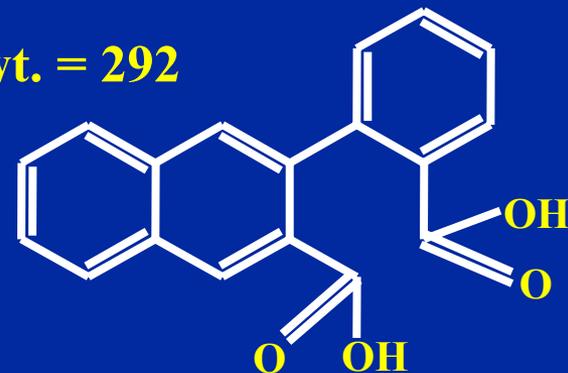
mol. wt. = 262



Ortho Cleavage

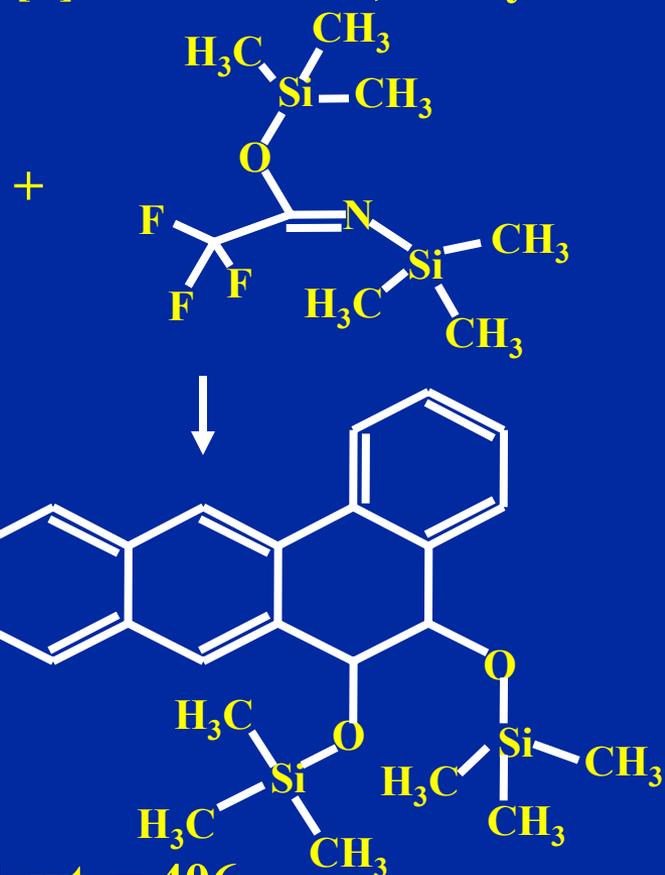


mol. wt. = 292



Benz[*a*]anthracene 5,6-dihydrodiol

3-[2-carboxylphenyl]-2-naphthoic acid



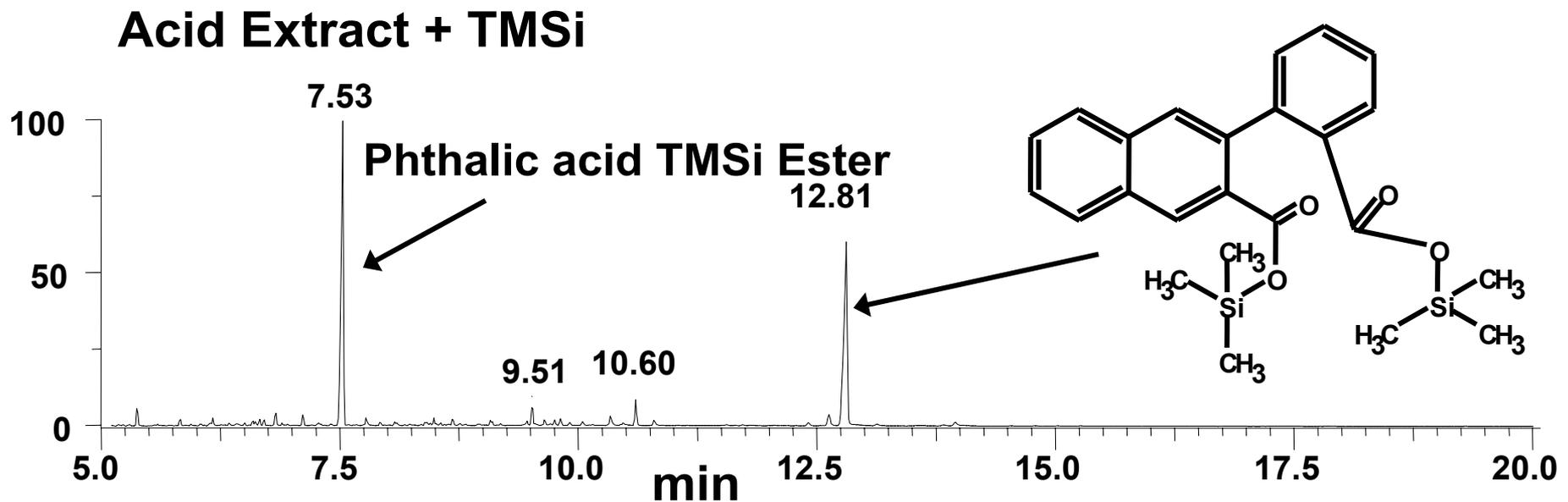
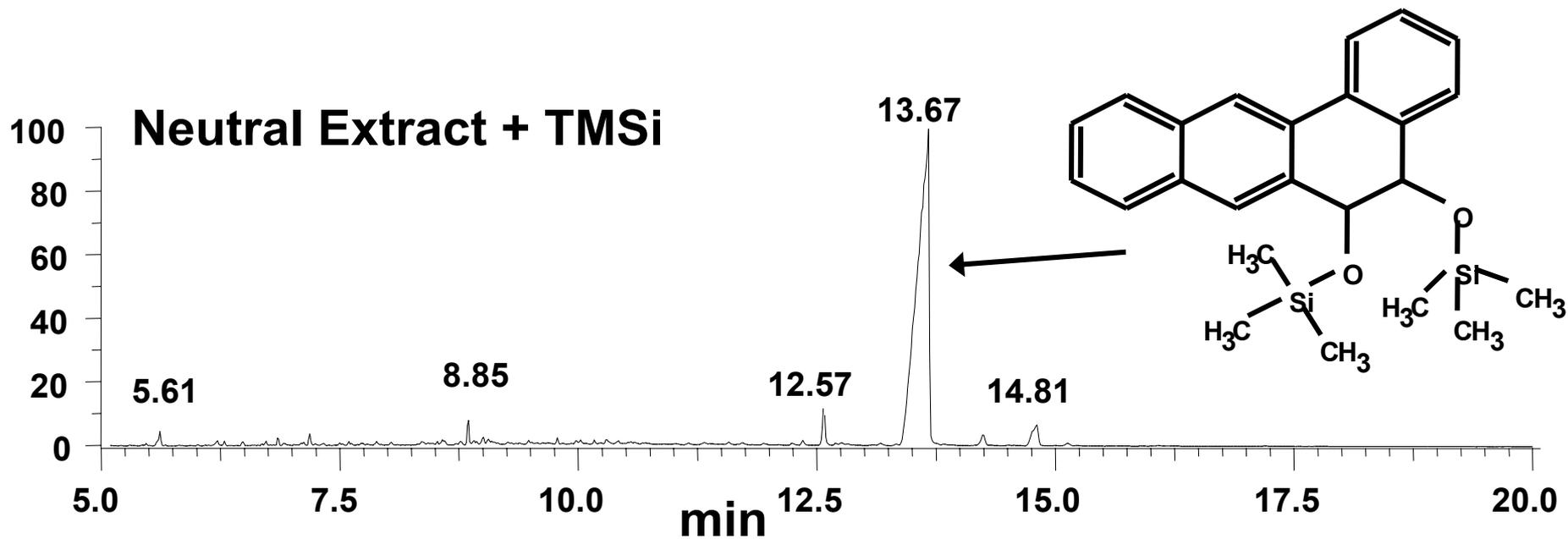
mol. wt. = 406

BSTFA  
(MW = 257)



mol. wt. = 436

# BA-5,6-Dihydrodiol Metabolism



**Macleod, C.T., and A.J. Daugulis, 2003. Biodegradation of polycyclic aromatic hydrocarbons in a two-phase partitioning bioreactor in the presence of a bioavailable solvent. *Appl. Microbiol. Biotechnol*, 62:291-296**

- **Macleod and Daugulis (2003) Queens University, Kingston, Canada, used *Mycobacterium* sp. PYR-1 in a two-phase partitioning bioreactor and determined phenanthrene and pyrene degradation.**
- **They found that PYR-1 degraded a gram of phenanthrene and pyrene in 4 days at a rate of 168 mg/l per day and 138 mg/l per day which is the highest reported in the literature.**

# SUMMARY AND CONCLUSIONS

## (Metabolism)

- *Mycobacterium vanbaalenii* PYR-1 is capable of degrading a wide variety of PAHs including naphthalene, anthracene, phenanthrene, fluoranthene, pyrene, benzo[*a*]pyrene, benz[*a*]anthracene and 7,12-dimethylbenz[*a*]anthracene.
- *M. vanbaalenii* PYR-1 is highly regio- and stereoselective in the degradation of PAHs.
- *M. vanbaalenii* PYR-1 initiates its attack on PAHs by dioxygenation and monooxygenation mechanisms.
- The reactions of dioxygenation at the K-region to form *cis*-dihydrodiols and *ortho* – cleavage to form PAH- dicarboxylic acid is characteristic in *Mycobacterium* species.
- These studies provides evidence for the potential application of this organism for improved PAH bioremediation and enantioselective production of dihydroxylated synthons

# Research Strategy

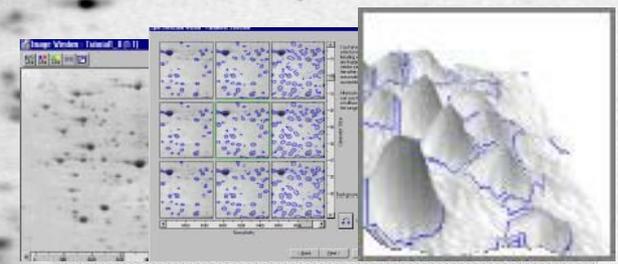
- Screen *Mycobacterium* sp. PYR-1 for ability to metabolize PAHs
- Elucidation of metabolic pathways
- Determine enzyme activity
- Purification and characterization of proteins
- Making and screening a cDNA library
- Molecular cloning and sequencing of the genes
- Over expression of the genes in *E. coli*
- Identification and confirmation of the gene and gene products

# NCTR Proteomics Work Flow

**Complex protein mixture**  
(e.g. cell lysate)

**Fractionate**

**SDS-PAGE or**  
**2D gel electrophoresis**



**Visualize / Image Gels**  
**(Fluorescent stains)**  
**Analyze Gels**  
**& Select Spots**



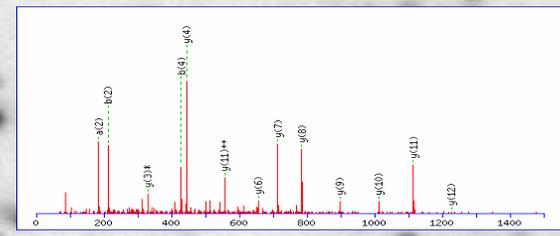
**Excise Protein**  
**Spots**



**In-Gel Digest /**  
**Prep for MS**

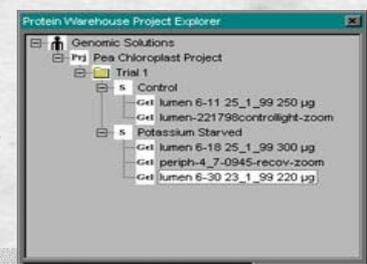


**NanoLC/MS/MS**  
**QTOF, Ion Trap**  
**MALDI TOF**



1 VLSADDKTNI KNCWGKIGGH GGEYGEEALQ RMFAAFPTTK TYFSHIDVP  
51 GSAQVKAHGK KVADALAKAA DHVEDLPGAL STLSDLHAHK LRVDPVNFKF  
101 LSHCLLVTLA CHHPGDFTP MHASLDKFLA SVSTVLTSKY R

**Identify / Characterize**  
**proteins**



**Database**  
**Archive / Retrieve**  
**Data**

# *Mycobacterium sp.* PYR-1 Cell Culture

Induced with 10 µg/ml PAH  
for 96 h, incubated for 120 h

Non-induced cells incubated  
for 120 h

**Induced cell pellet**

**Cell lysis (Supernatant)**  
2DE (Amersham)  
Differential expression analysis

**Non- Induced cell pellet**

Spot excision (ProPic, GSI)  
In-gel digestion (ProGest, GSI)  
Nano LC/MS/MS (Q-ToF API US, Micromass)

Mascot (Matrix Science)

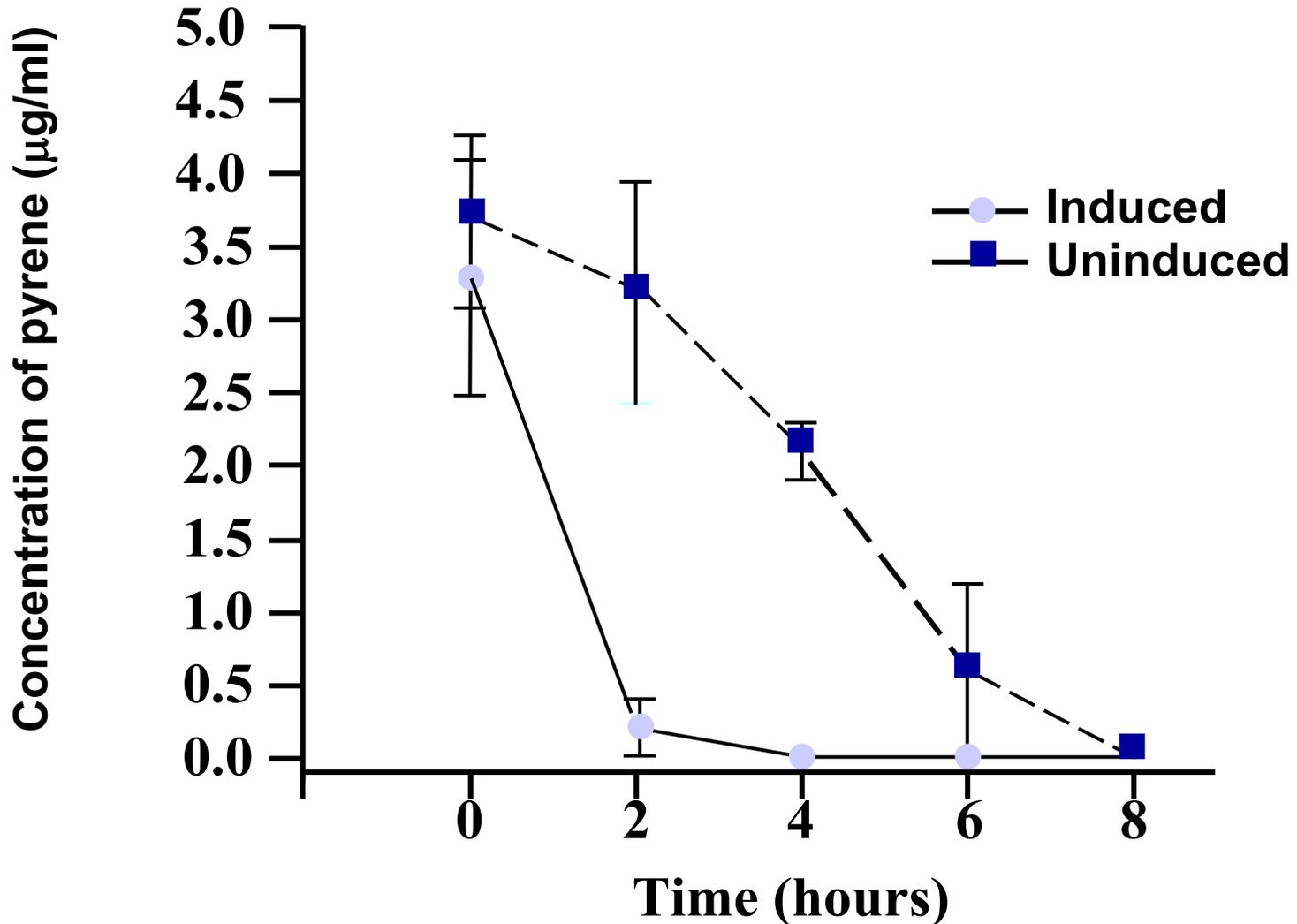
**Match by identity  
to another species**

**Tentative match by  
identity to another species**

**No match**

*De novo* sequencing  
Homology searching:  
**MS-BLAST (EMBL)**  
**MS-Homology (UCSF)**

# Effect of Induction of Pyrene Metabolism in Cells of *Mycobacterium* sp. PYR-1



kDa

200

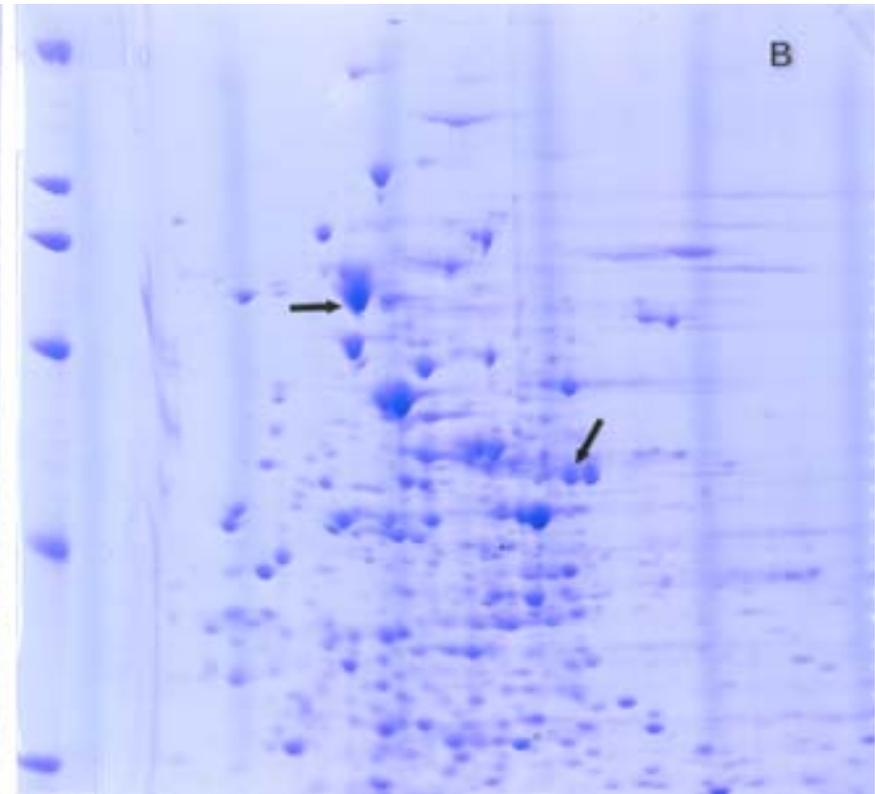
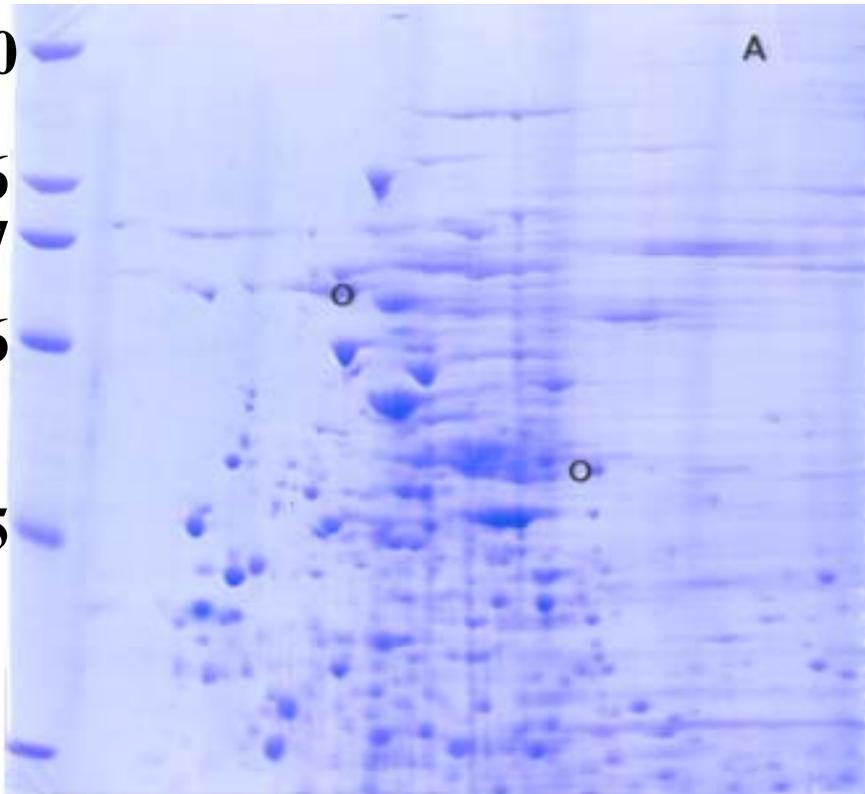
116

97

66

45

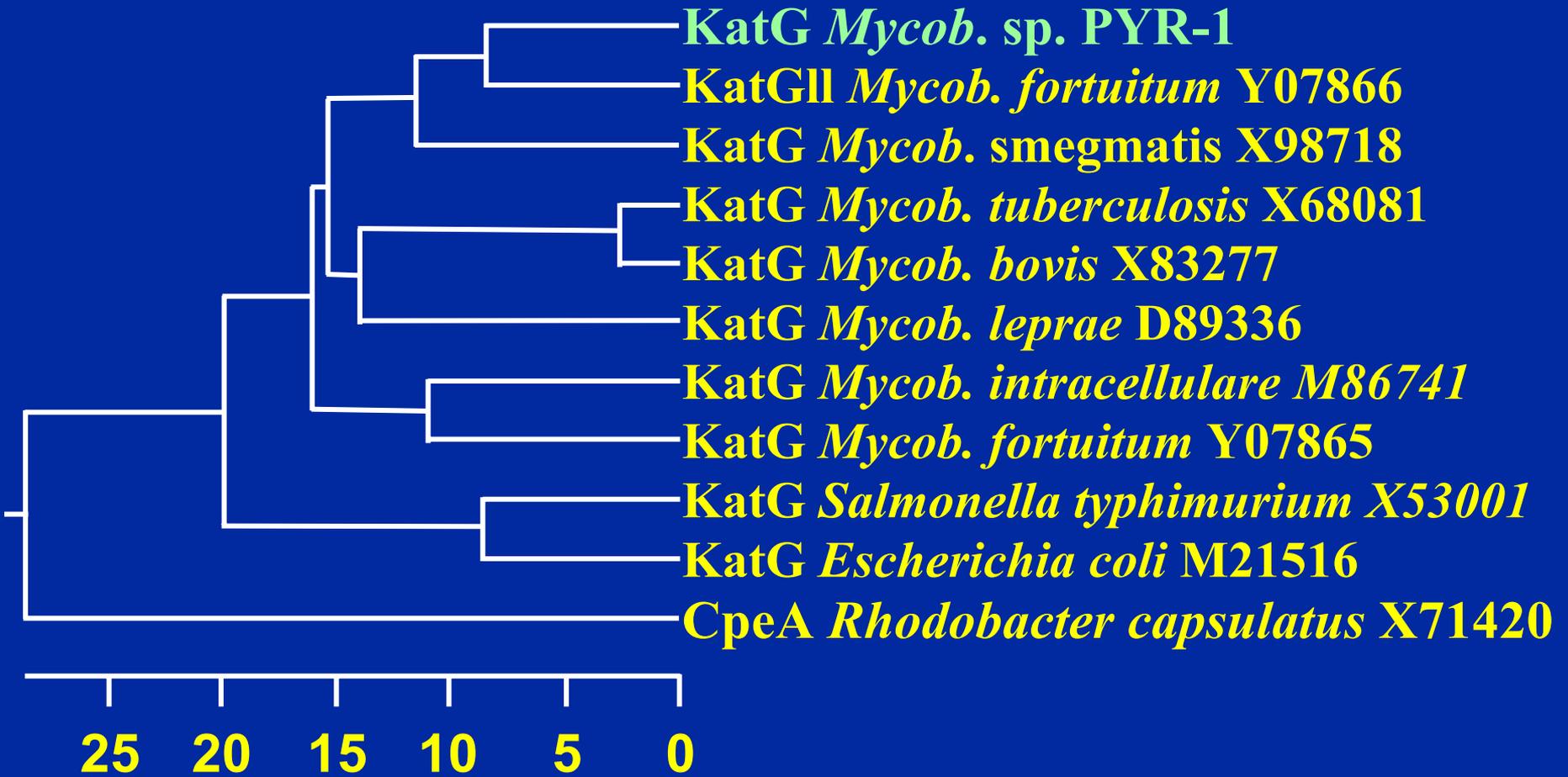
31



**Un-induced**

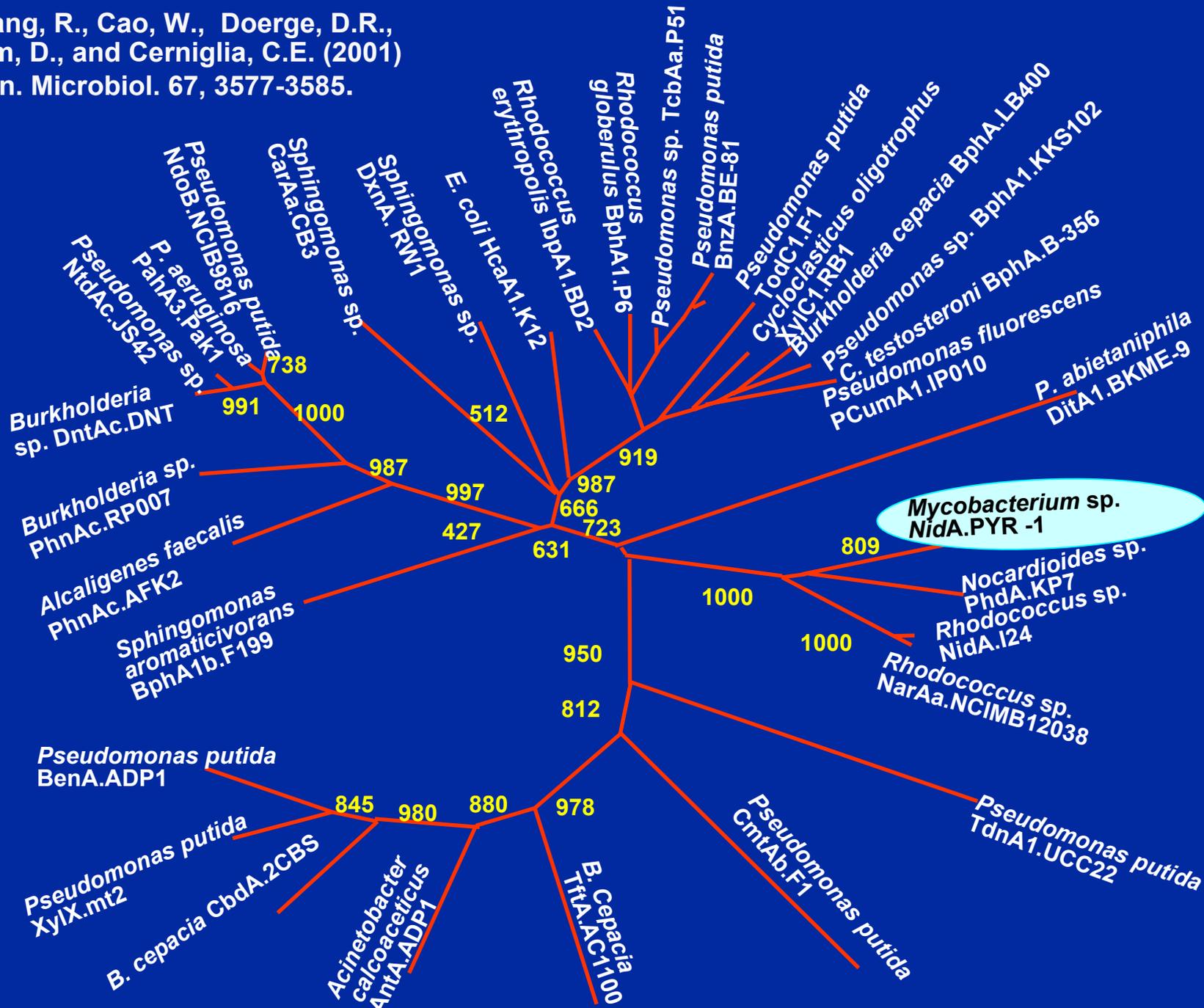
**Induced with pyrene**

**2D gel for identification of PAH-induced proteins of *Mycobacterium* sp. Strain PYR-1.**

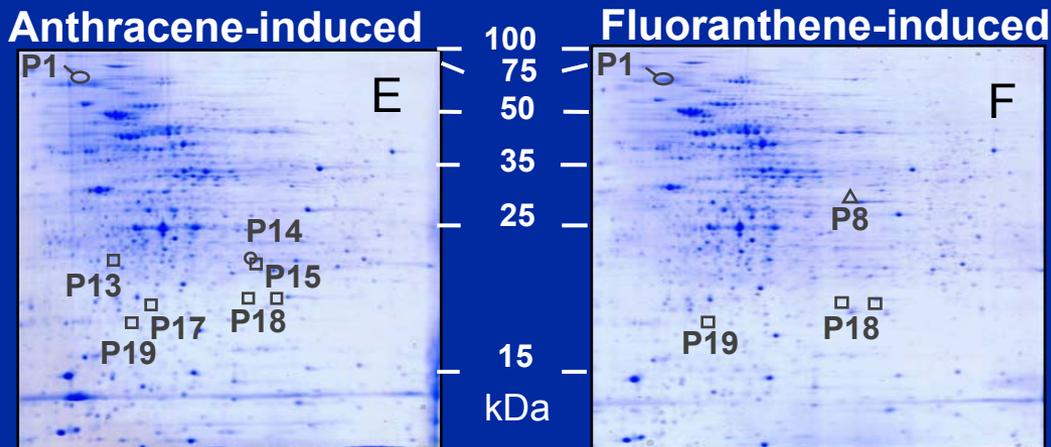
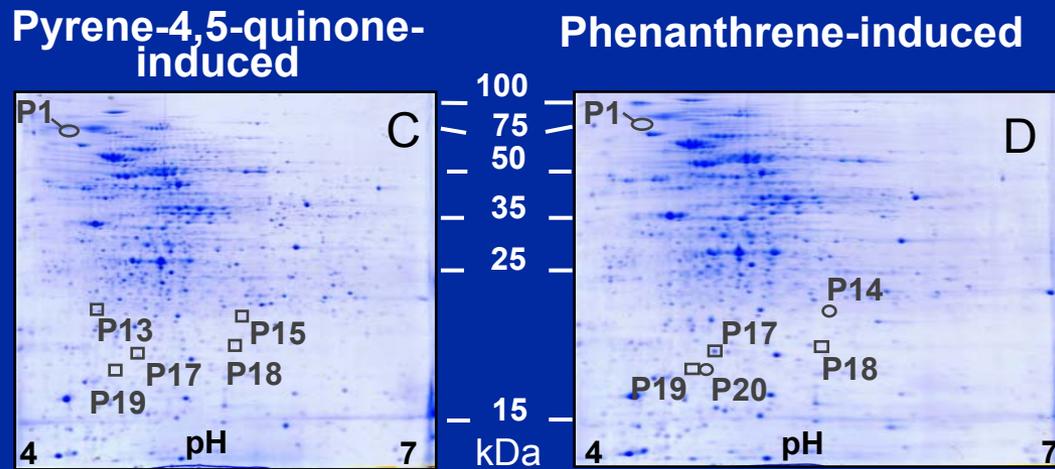
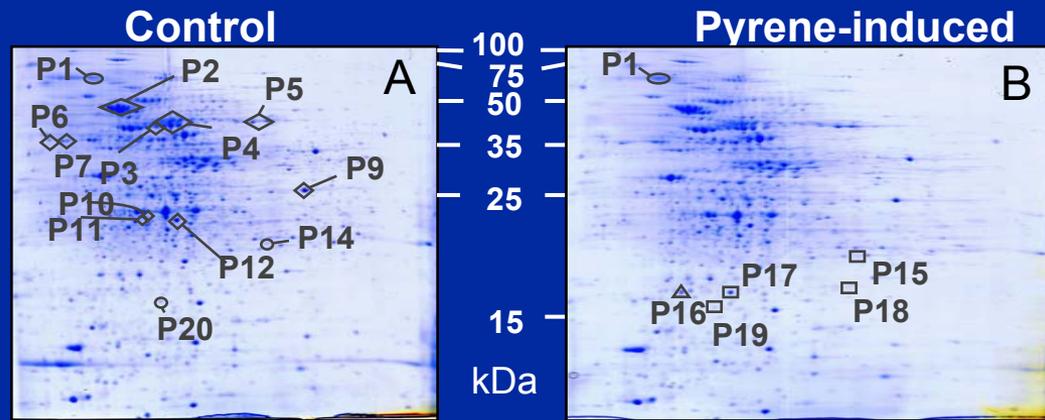


Wang, R. Wennerstrom, D., Cao, W., Khan, A., and Cerniglia, C. (2000) *Appl. Environ. Microbiol.* 66, 4300-4304.

Khan, A., Wang, R., Cao, W., Doerge, D.R.,  
Wennerstrom, D., and Cerniglia, C.E. (2001)  
Appl. Environ. Microbiol. 67, 3577-3585.

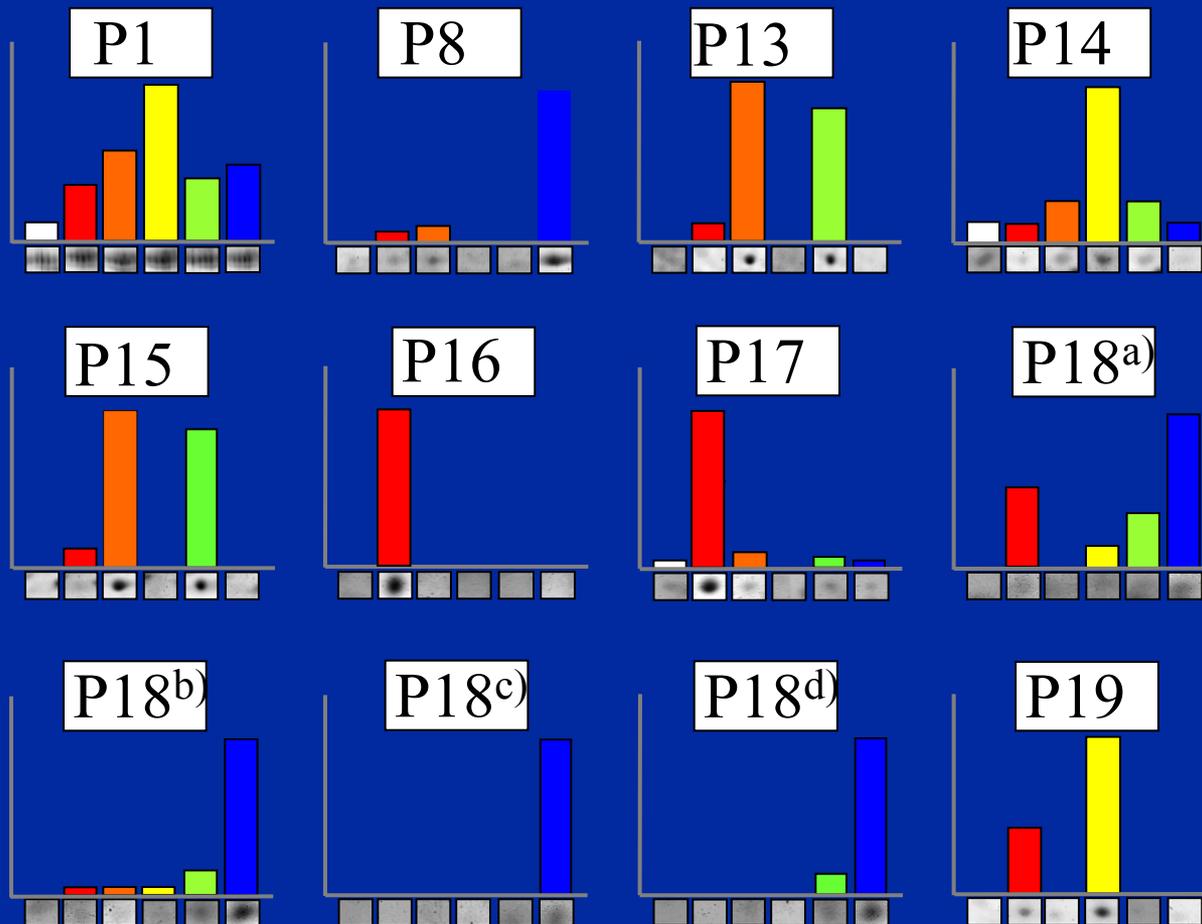


# Protein expression profiles from PYR-1



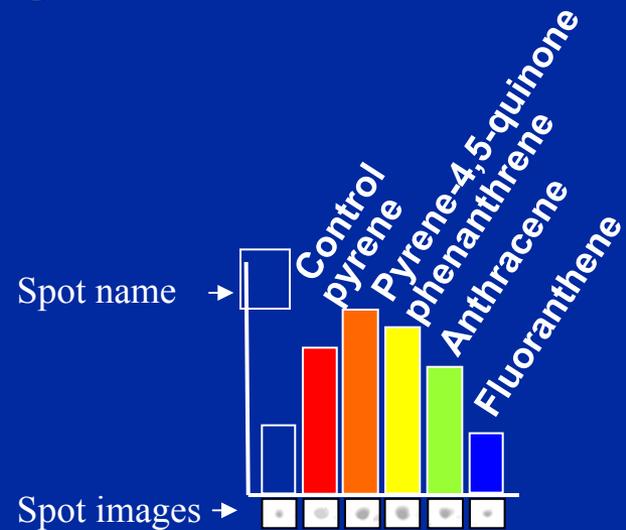
Kim, S.-J., Jones, R.C., Cha, C.-J., Kweon, O., Edmondson, R.D., and Cerniglia, C.E. 2004. Proteomics. (In Press)

# Relative Synthesis Rate Of Up-regulated Proteins During Induction



Synthesis rates are normalized to total spot volume.

The conditions are defined in the lower right. The six insets below each bar plot show spot images of the corresponding 2-D gels.



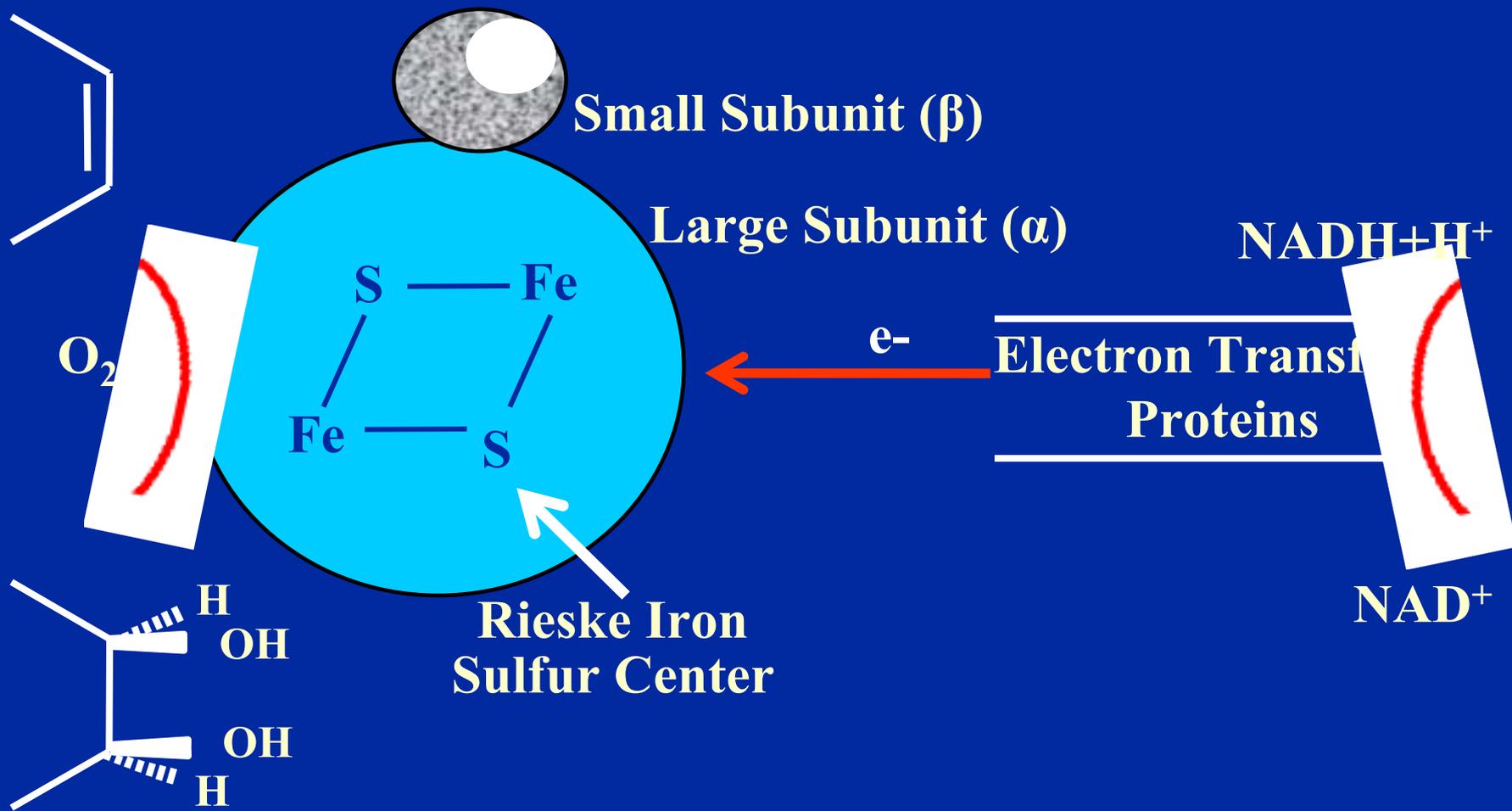
# Identified Functions of PAH-Induced Polypeptides

Protein No.	Protein description	Species	Accession No.	Peptides Matched	Observed migration		Theoretical migration	
					M (kDa)	p/	M (kDa)	p/
P1	Catalase-peroxidase KatG	<i>M. vanbaalenii</i> PYR-1	AAF20142	41	8.52	4.54	80.9	4.53
P2	Heat shock protein 65	<i>M. marinum</i> ATCC927	AAN62885	53	60.7	4.75	57.4	4.90
P3	ATP synthase [beta] chain Putative magnesium chelatase	<i>M. leprae</i> TN	NP_301839	18	51.1	5.01	53.1	4.90
		<i>M. tuberculosis</i> CDC1551	NP_335419	4			49.6	5.20
P4	Fumarase Aldehyde dehydrogenase 6-phosphogluconate dehydrogenase (decarboxylating)	<i>M. leprae</i> TN	NP_302313	19	519	5.07		
		<i>M. vanbaalenii</i> PYR-1	AAF75990	7			50.3	5.69
		<i>M. tuberculosis</i> CDC1551	NP_336349	7			52.0	4.95
							51.5	5.31
P5	Hypothetical protein Rv0462	<i>M. tuberculosis</i> H37Ru	NP_214976	3	53.6	5.67	49.4	5.66
P6	Heat shock cognate protein	<i>Cricetulus griseus</i>	P 19378	39	58.1	4.26	71.0	5.15
P7	Probable enolase	<i>M. tuberculosis</i> H37 Ru	NP_215539	10	44.9	4.34	45.0	4.37
P8	No match				44.4	5.95		
P9	35-1od antigen	<i>M. tuberculosis</i> H37 Ru	NP_217260	16	41.2	6.14	29.2	5.81
P10	Unnamed protein product	<i>M. tuberculosis</i> Erdmann	CAA45101	6	25.5	4.93	44.4	5.19
P11	Indole-3-glycerolphosphate synthase Indole-3-glycerolphosphate synthase	<i>Pseudomonas putida</i>	P20578	4	25.1	4.90	30.6	4.92
		<i>M. leprae</i> TN	NP_301916	4			28.4	5.14
P12	Probable elongation factor Tsf	<i>M. tuberculosis</i> H37 Ru	NP_217405	7	25.1	5.16	28.9	5.18
P13	No match				219	4.71		
P14	No match				223	5.80		
P15	Putative monooxygenase YcdM	<i>Escherichiacob</i>	P75898		219	5.85	39.9	5.07
P16	No match				18.6	4.70		
P17	No match				18.6	5.08		
P18	Homologous to iron-sulfur proteins	<i>Rhodococcus</i> sp. CIR2	BAA76339		19.0	5.78	19.8	4.55
P19	Dioxygenase small subunit NidB	<i>M. vanbaalenii</i> PYR-1	AAF75992	14	18.0	4.98	19.4	4.87
P20	No Match				18.0	5.04		

# SUMMARY AND CONCLUSIONS

## (Proteomics)

- *M. vanbaalenii* PYR-1 has an inducible system for PAH degradation.
- More than 1000 gel separated proteins within pI of 4-7 and Mr of 10-100 kDa were detected.
- A number of proteins were shown to be over expressed when *M. vanbaalenii* PYR-1 was induced with PAHs.
- Several conditions – specific marker proteins seems to be uniquely over-expressed when *M. vanbaalenii* PYR-1 was induced with various PAHs.
- At least 8, 6, 6, 7 and 4 proteins from pyrene-, P45Q, phenanthrene, anthracene and fluoranthene, respectively were shown to be induced when compared to uninduced control sample.

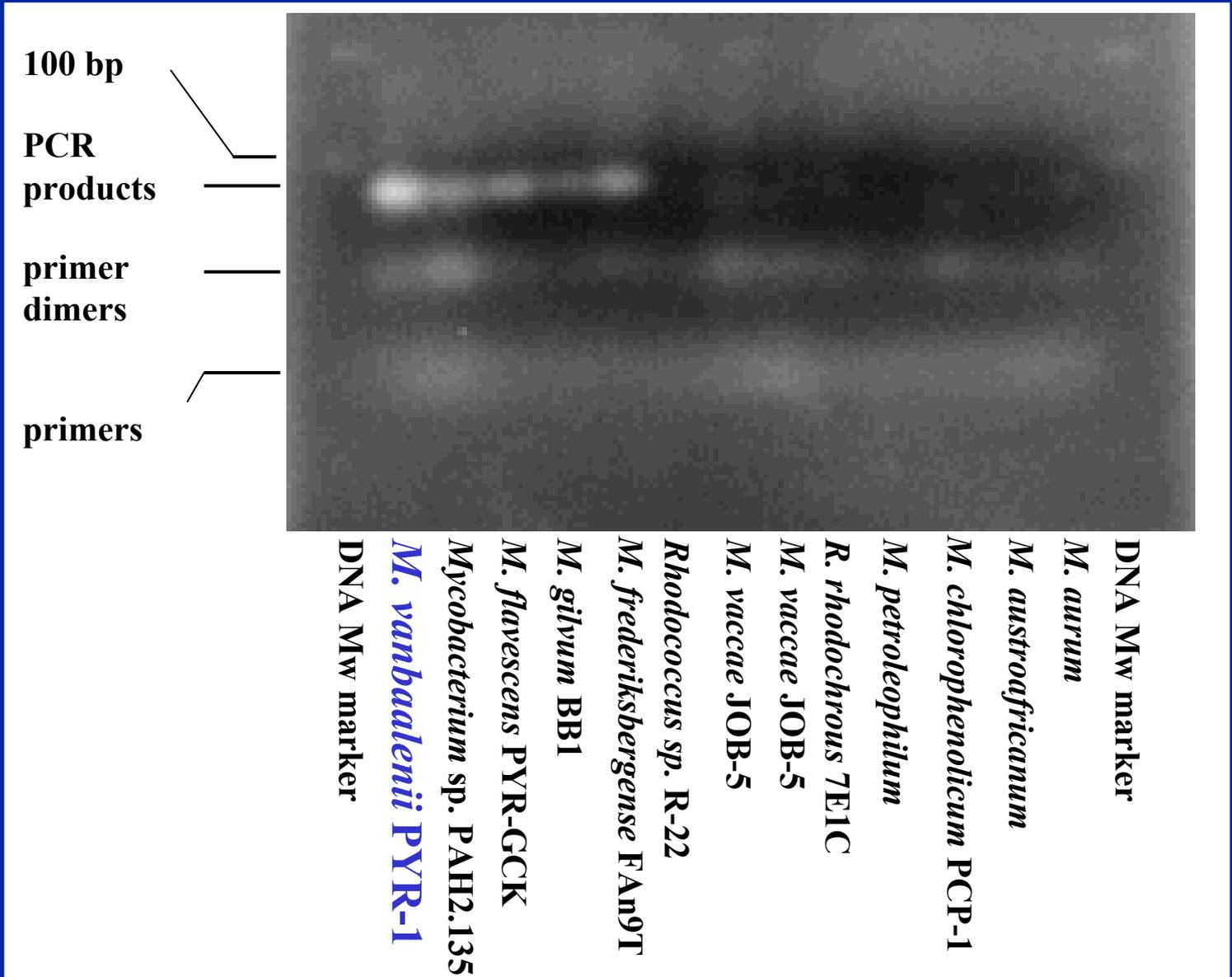


**Initial dioxygenase attack on an aromatic hydrocarbon substrate**

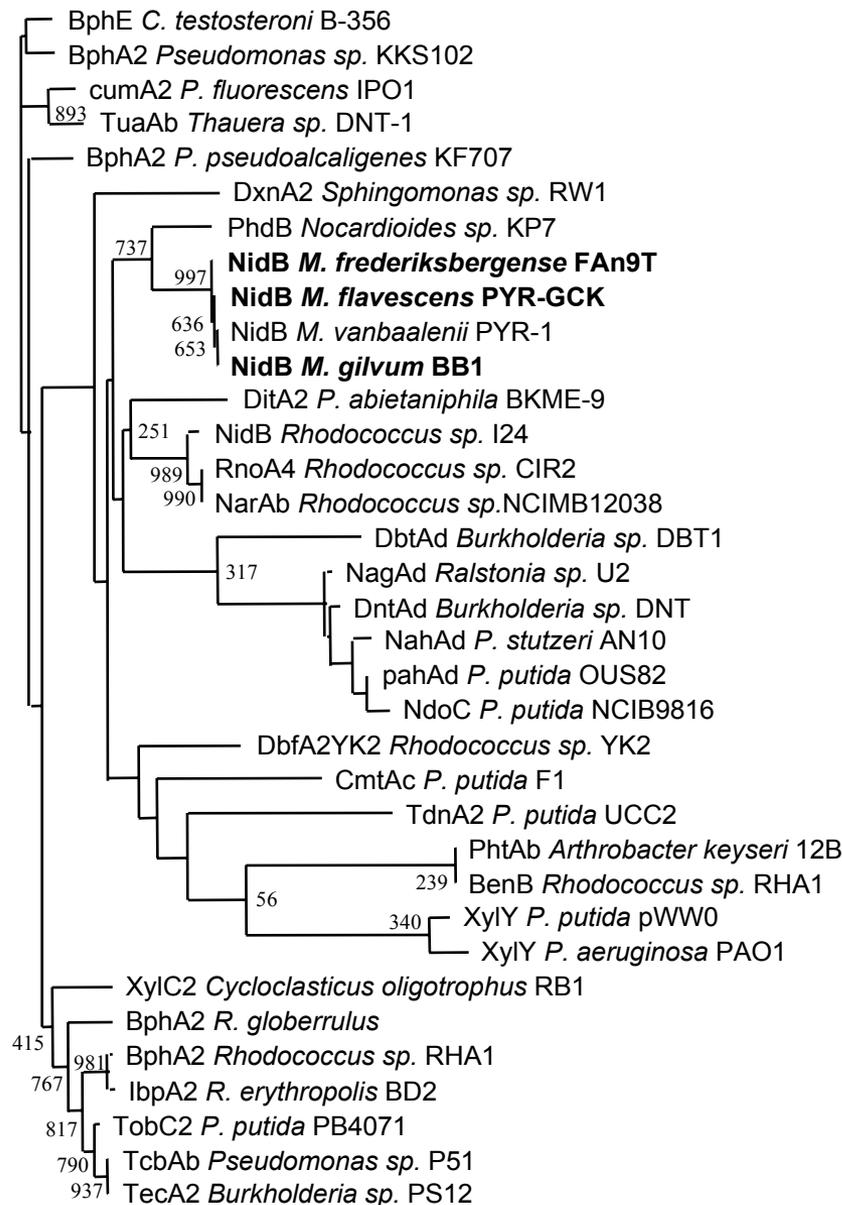
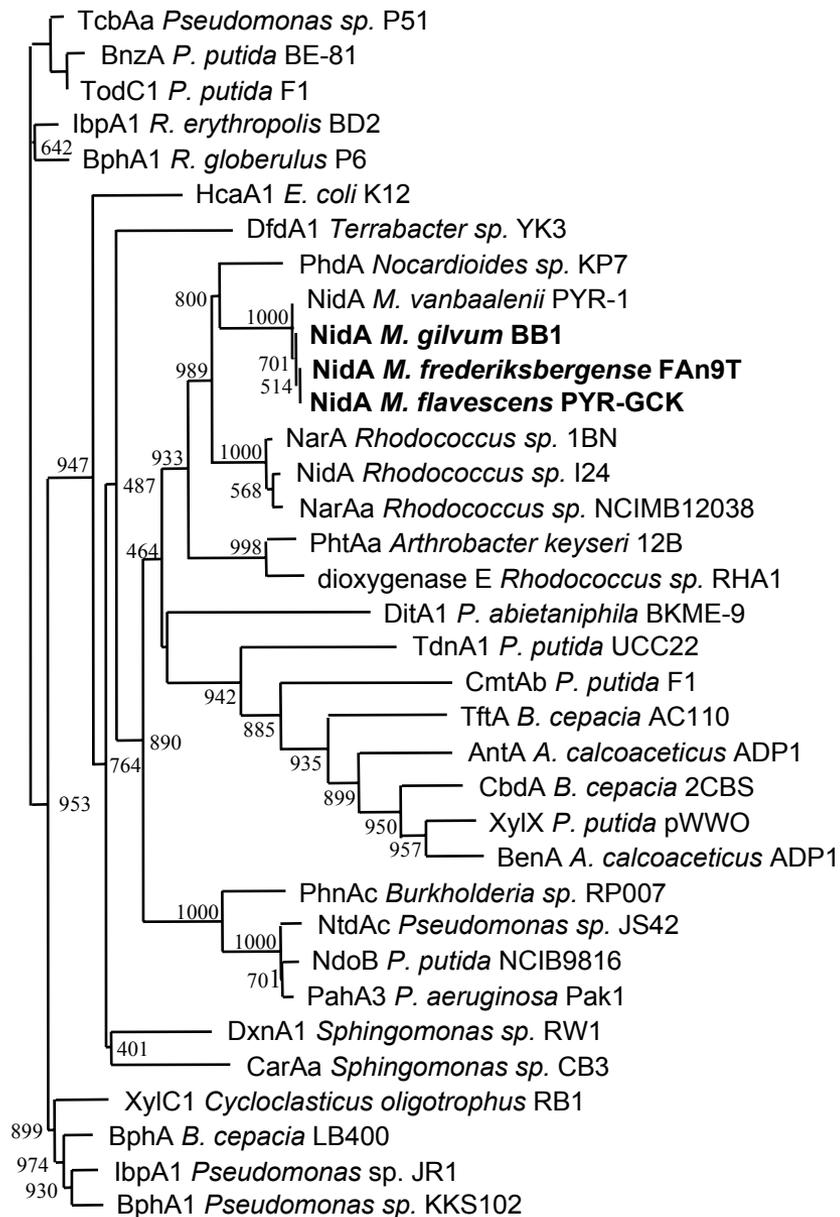
# N-terminal and Central Part of the $\alpha$ Subunits of Ring-hydroxylating Dioxygenase

<i>Mycobacterium</i> sp. PYR1.NidA	97	CRHRGALVCRAEMGNTAHFQCPYH	120-211	NWKTAADNFVGD SYHT-LFAH	230
<i>Nocardioides</i> sp. KP7.PhdA	91	CRHRGTLPCRTEAGNTSHFRCPYH	114-205	NWKL GADNFVGD DAYHT-LMTH	224
<i>P. putida</i> NCIB9816.NdoB	81	CRHRGKTLVSVEAGNAKGFVCSYH	104-194	NWKAPAENFVGD DAYHV-GWTH	213
<i>Rhodococcus</i> sp. I24.NidA	88	CRHRGMQVCRAEMGNASHFRCPHH	111-202	NWKL GADNFVGD DAYHT-MMTH	221
<i>Sphingomonas</i> sp. CB3.CarAa	75	CRHRGGALCRGESGNTKNFICTYH	98-189	NWKLA AEQFTTDDDFHF-LTSH	208
<i>Sphingomonas</i> sp. RW1.DxnA1	85	CRHRGNRLCLADRGNAKSFRC SYH	108-195	NWKWQAEQHATDHLHV-AVSH	214
<i>P. abietaniphila</i> BKME-9.DitA1	91	CPHRGMRISTADCGNTQIHKCITH	114-205	NWKTAGEQSAADGFHT-LTLH	224
<i>P. putida</i> BE-81.BnzA	96	CRHRGMRICRADAGNAFAFTCSYH	119-208	NWKFAAEQFCSDMYHAGTTSH	228
<i>B. cepacia</i> LB400.BphA	100	CRHRGMRICRSDAGNAKAFTCSYH	123-219	NWKFAAEQFCSDMYHAGTTTH	239
<i>P. putida</i> UCC22.TdnA1	94	CSHRGASVCREHRGNAAGFTCPYH	117-208	NWKL VWDNAG-DGYHV-PFSH	226
<i>P. putida</i> F1.CmtAb	84	CPHRGATVCRERSGNSKNFQCFYH	107-198	NWKLLVENS I-DGYHA-VSTH	216
<i>P. putida</i> mt2.XylX	92	CSHRGATLCRERSG NKATH TCSFH	115-208	NWKVQV ENGA-DGYHV-STVH	226

# PCR Detection of Rieske Center

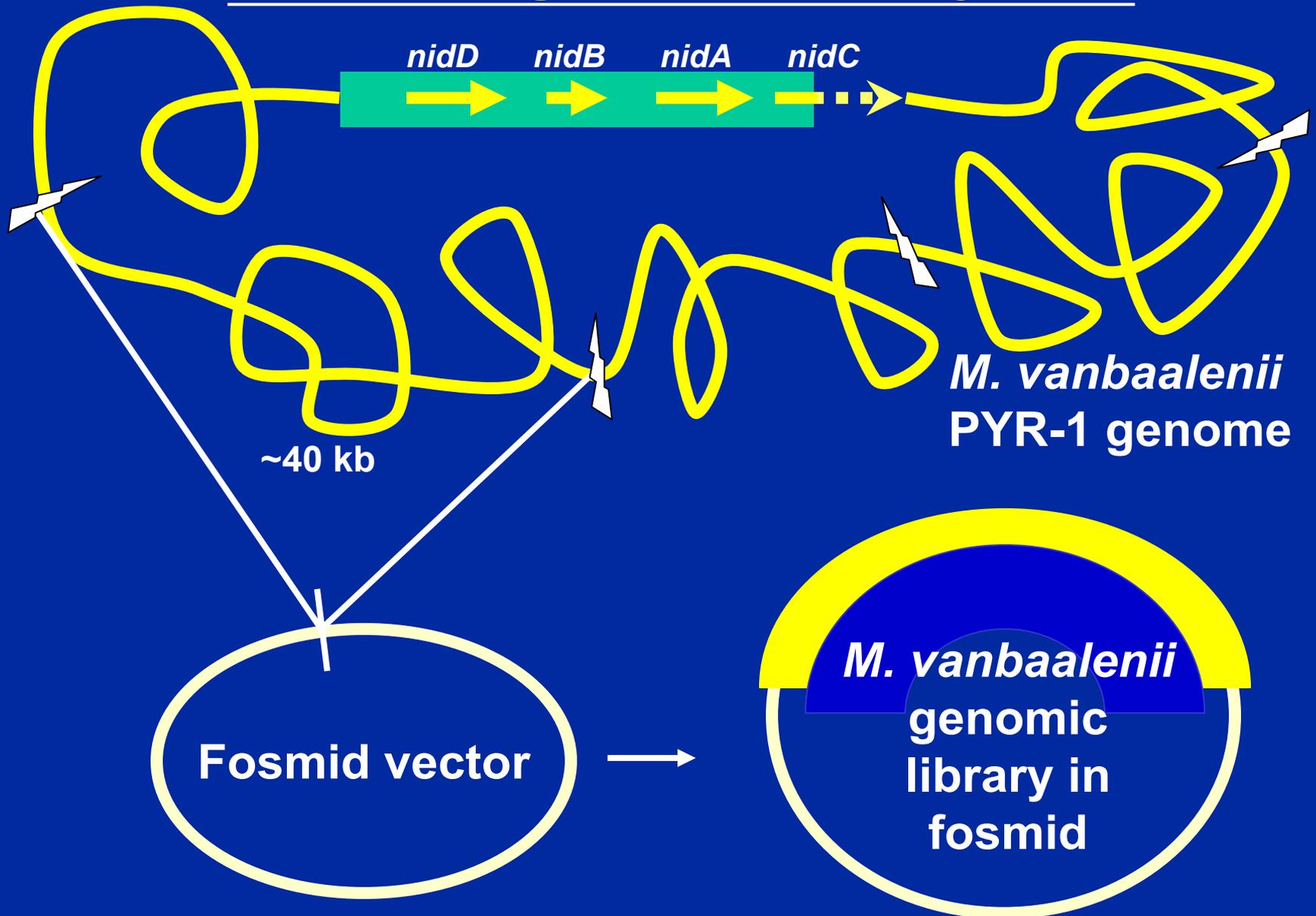


Bacterial Strain	Isolation Source	Plate spraying test <sup>b</sup>		PCR			Southern hybridization <sup>e</sup>	
		Phe	Pyr	Rieske <sup>c</sup>	<i>nidA</i>	<i>nidB</i>	<i>nidA</i>	<i>nidB</i>
<i>M. vanbaalenii</i> PYR-1 [DSM 7251]	oil contaminated sediment, Texas	+	+	+	+	+	+	+
<i>Mycobacterium</i> sp. PAH 2.135 (RJGII135)	coal gasification site soil, Illinois	+	+	+	-	+	+	+
<i>M. flavescens</i> PYR – GCK [ATCC 700033]	Polluted sediment, Indiana	+	+	+	+	+	+	+
<i>M. gilvum</i> BB1 [DSM 9487]	former coal gasification site, Germany	+	+	+	+	+	+	+
<i>M. frederiksbergense</i> FAn9T [DSM 44346]	coal tar contaminated soil, Denmark	+	+	+	+	+	+	+
<i>Rhodococcus</i> sp.R-22 [ATCC 29671]	Soil	-	-	-	-	-	-	-
<i>M. vaccae</i> JOB-5 [ATCC 29678]	Soil	-	-	-	-?	-?	-	-
<i>Mycobacterium</i> sp. 7E1B1W [ATCC 29676]	Soil	-	-	-	-	-	-	-
<i>R. rhodochrous</i> 7E1C [ATCC 19067]	Soil	-	-	-	-	-	-	-
<i>M. petroleophilum</i> [ATCC 21497]	drilling well	-	-	-	-?	-?	-	-
<i>M. chlorophenicum</i> PCP-1 [ATCC 49826]	paper industry polluted sediment, Finland	-	-	-	-	-	-	-
<i>M. austroafricanum</i> [ATCC 33464]	soil, south Africa	-	-	-	-?	-	-	-
<i>M. aurum</i> [ATCC 23366]	Soil	-	-	-	-	-	-	-

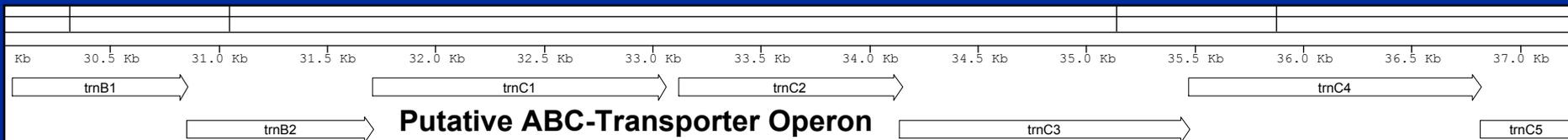
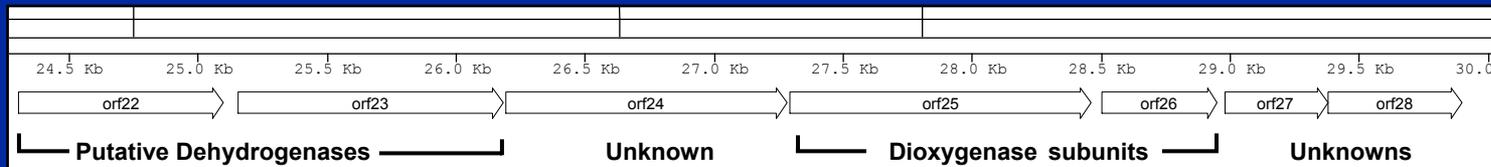
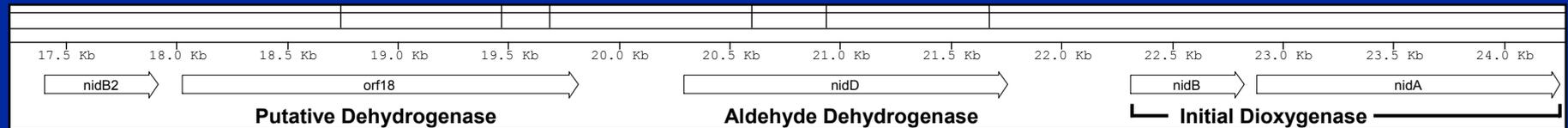
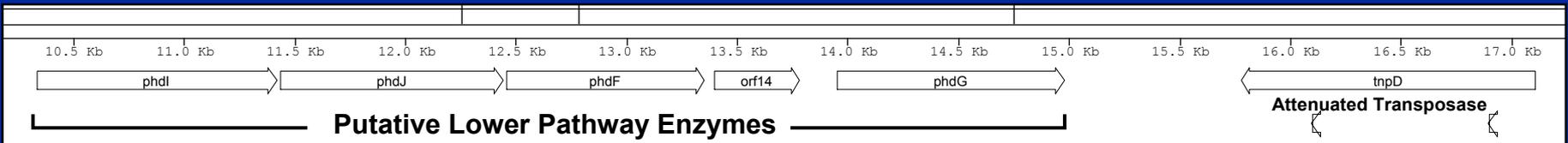
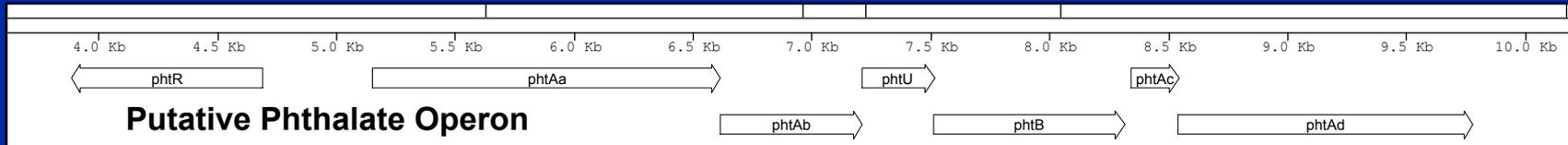
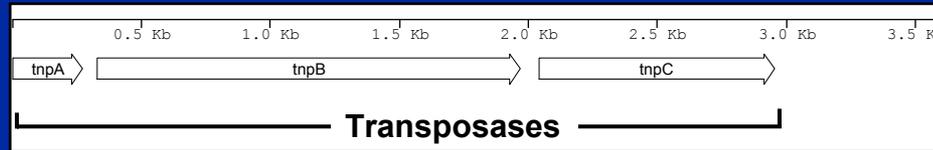


# Phylogenetic Trees Of Dioxygenase Subunits

# Creating Fosmid Library



# Putative Genes in Fosmid Clone



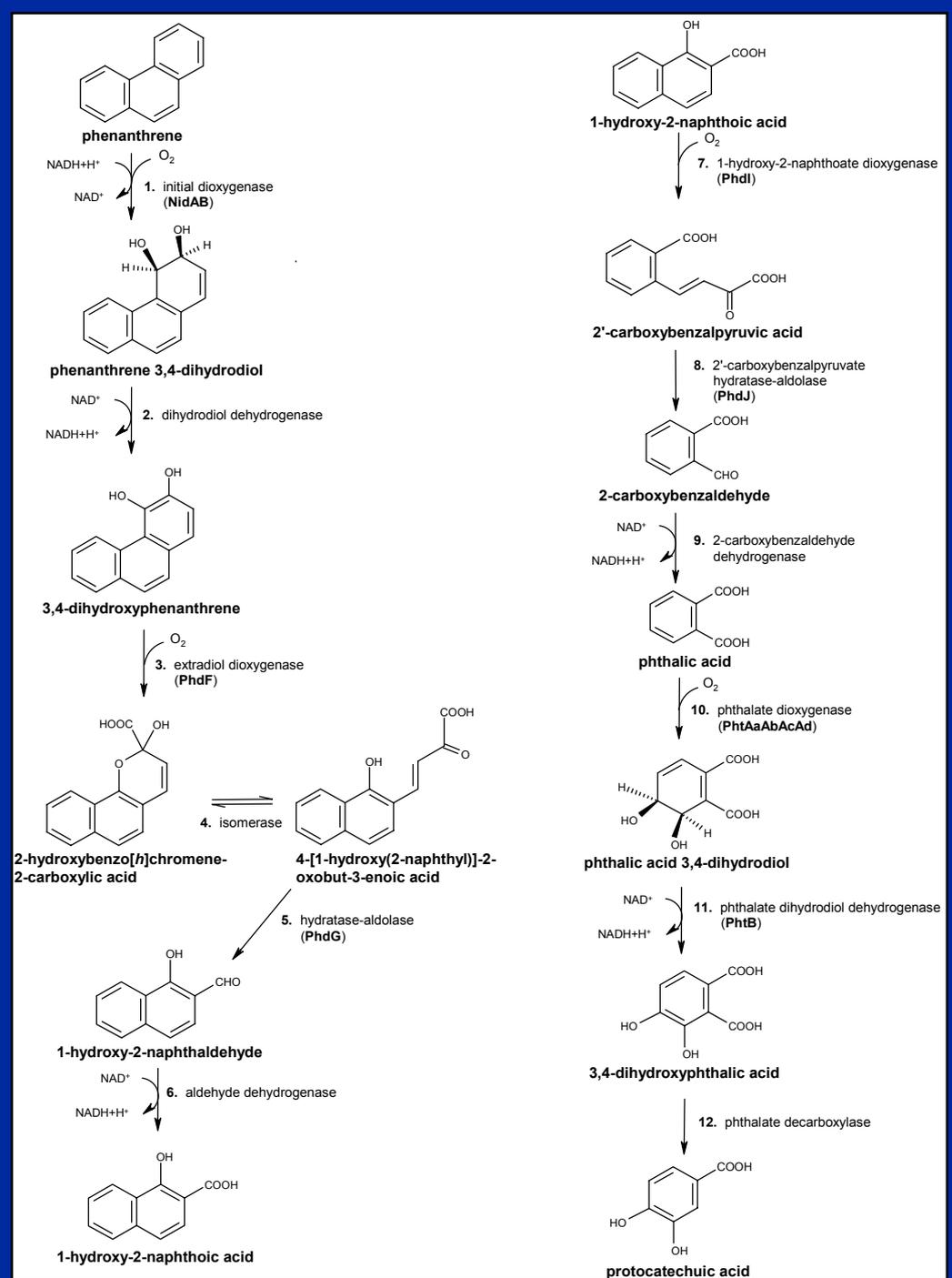
# Proposed *M. vanbaalenii* PYR-1 Phenanthrene Degradation Pathway

## Putative Genes for:

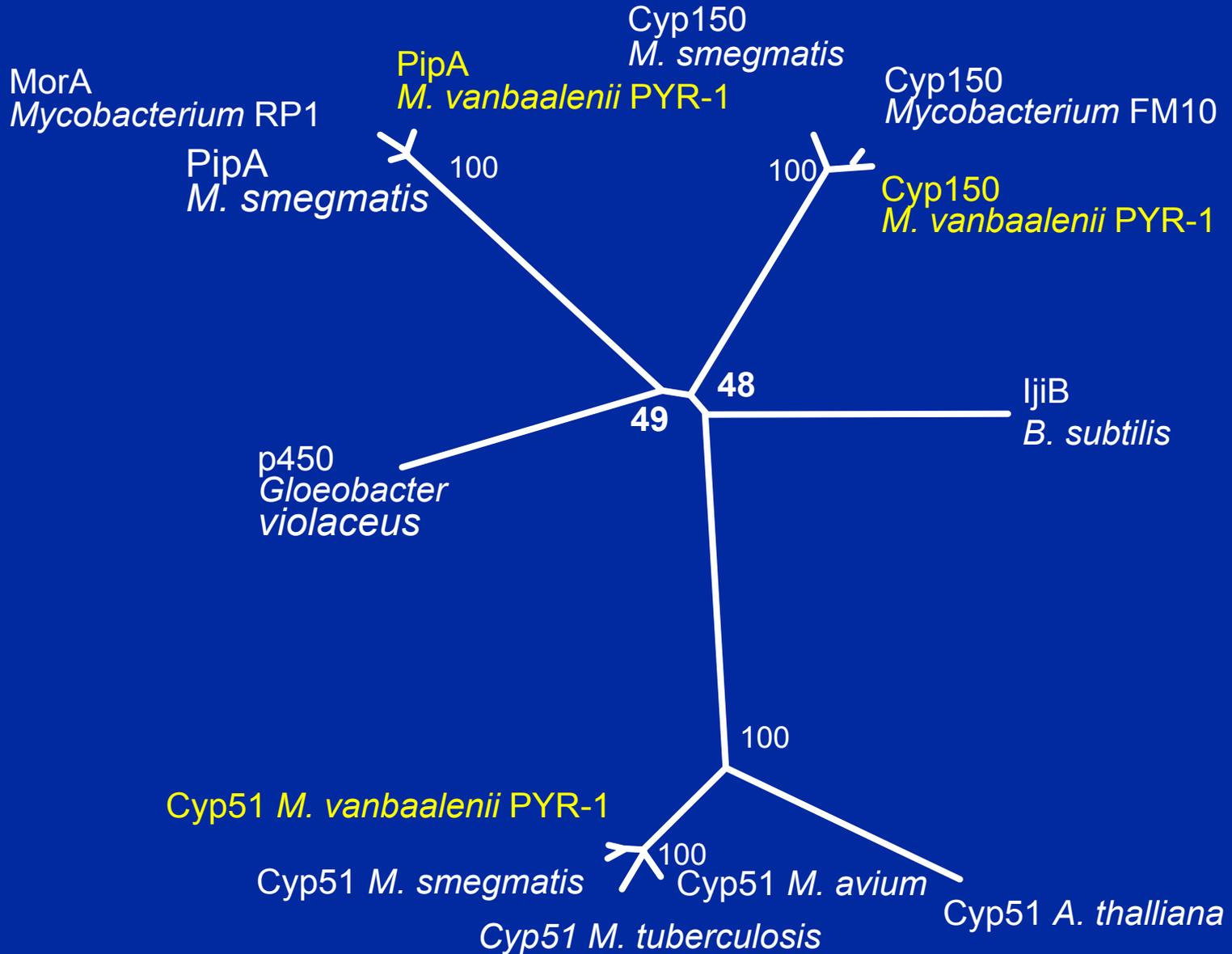
1. Initial dioxygenase (*nidAB*)
2. Dehydrogenase
3. Extradiol dioxygenase (*phdF*)
4. Isomerase
5. Hydratase-aldolase (*phdG*)
5. Dehydrogenase
6. Dioxygenase (*phdI*)
7. Hydratase-aldolase (*phdJ*)
8. Dehydrogenase
9. Phthalate dioxygenase (*phtAaAbAcAd*)
10. Phthalate dihydrodiol dehydrogenase (*phtB*)
11. Decarboxylase

Stingley, R.L., Khan, A.A., and Cerniglia, C.E. (2004). *Biochem. Biophys. Res. Commun.* 322:1133-146.

Stingley, R.L., Brezna, B., Khan, A.A., and Cerniglia, C.E. (2004) *Microbiol* (In Press)



# Phylogenetic Tree of Three Cytochrome P450's from *Mycobacterium vanbaalenii* PYR-1



## **Mycobacterium Strains That Were Screened For The Presence Of *nidA* and *nidB* and Cytochrome P450 Genes**

Strain	Substrate or other characteristic	Detection of				
		<i>nidA</i> <sup>c</sup>	<i>nidB</i> <sup>d</sup>	<i>pipA</i> <sup>e</sup>	<i>cyp150</i> <sup>f</sup>	<i>cyp51g</i>
<i>M. aurum</i> [ATCC 23366]	Type strain	-	-	+	+	+
<i>M. austroafricanum</i> [ATCC 33464]	Type strain, related to <i>M. vanbaalenii</i>	-	-	+	+	+
<i>M. austroafricanum</i> GTI-23 <sup>a</sup>	PAHs	+	+	+	-	-
<i>M. chlorophenicum</i> PCP-1 [ATCC 49826]	Polychlorinated phenols	-	-	+	-	+
<i>M. flavescens</i> PYR – GCK [ATCC 700033]	PAHs	+	+	+	+	-
<i>M. frederiksbergense</i> FAn9T [DSM 44346]	PAHs	+	+	+	+	+
<i>M. gilvum</i> [ATCC 43909]	Type strain	-	-	+	+	-
<i>M. gilvum</i> BB1 [DSM 9487]	PAHs	+	+	+	+	-
<i>M. petroleophilum</i> [ATCC 21497]	n-paraffins	-	-	+	+	+
<i>M. smegmatis</i> mc2155 [ATCC 700084]	Transformation host	-	-	+	+	+
<i>M. vaccae</i> JOB-5 [ATCC 29678]	Gaseous, long chain, cycloparaffinic and monoaromatic hydrocarbons	-	-	+	+	+
<i>M. vanbaalenii</i> PYR-1 [DSM 7251]	PAHs	+	+	+	+	+
<i>Mycobacterium</i> sp. 7E1B1W [ATCC 29676]	Gaseous and long chain hydrocarbons	-	-	+	+	-
<i>Mycobacterium</i> sp. PAH 2.135 (RJGII-135) <sup>b</sup>	PAHs	+	+	+	-	+

<sup>a</sup>Obtained from Dr. B. W. Bogan at the Gas Technology Institute in Des Plaines, Illinois; <sup>b</sup>from Dr. D. Warshawsky at the University of Cincinnati. <sup>c</sup>PCR primers *nidAf* and *nidAr* were used; <sup>d</sup>primers *nidBf* and *nidBr*; <sup>e</sup>cumulative results from PCR with primers RP1F1 and RP1R2, RP1F2 and RP1R1, RP1F2 and PipAR2; <sup>f</sup>primers FM10F1 and FM10R2, <sup>g</sup>primers Cyp51F and CYP51R “+” PCR product of expected size was present, “-“ PCR product of expected size was not obtained.

# SUMMARY AND CONCLUSIONS

## (Genomics)

- PAH degradation enzymes in *M. vanbaalenii* PYR-1 are not encoded on plasmids.
- Genomic studies indicate that *M. vanbaalenii* PYR-1 has novel dioxygenases. No DNA hybridization was detected with well characterized toluene, naphthalene, xylene, and biphenyl dioxygenase.
- Different geographical origin of the studied strains indicates wide distribution of *nidA* and *nidB* genes in the environment and supports the usage of *M. vanbaalenii* PYR-1 as a model strain.
- The genes for the degradation of PAHs from *M. vanbaalenii* PYR-1 have been cloned, expressed and sequenced.

# SUMMARY AND CONCLUSIONS

## (Genomics Cont.)

- Most of the genes required for phenanthrene degradation are clustered together within 37 kb on the *M. vanbaalenii* PYR-1 genome
- PYR-1 has a number of different dioxygenase and cytochromic P-450 genes
- The PYR-1 phthalate degradation operon is functional
- The PAH-degradation genes examined thus far are constitutively expressed at the mRNA level, but may be induced by PAHs

# ***Mycobacterium species* PAH Degradation Studies**

## **Microbiology (NCTR)**

Barbara Brezna  
Wei-Wen Cao  
Chang-Jun Cha, Ph.D.  
Michael Heitkamp, Ph.D.  
Allison Henderson  
Ashraf A. Khan, Ph.D.  
Saeed A. Khan, Ph.D.  
Eungbin Kim, Ph.D.  
Seong-Jae Kim, Ph.D.  
Yong-Hak Kim, Ph.D.  
Oh-Gew Kweon, Ph.D.  
Joanna D. Moody  
Lisa Mullis  
Donald D. Paine  
Fateme Rafii, Ph.D.  
Debra Ross, Ph.D.  
Roger S. Steele  
Robin Stingley, Ph.D.  
Kidon Sung, Ph.D.  
John B. Sutherland, Ph.D.  
Rong-Fu Wang, Ph.D.

## **Biochem Tox (NCTR)**

Mona Churchwell  
Daniel R. Doerge, Ph.D.  
Peter P. Fu, Ph.D.

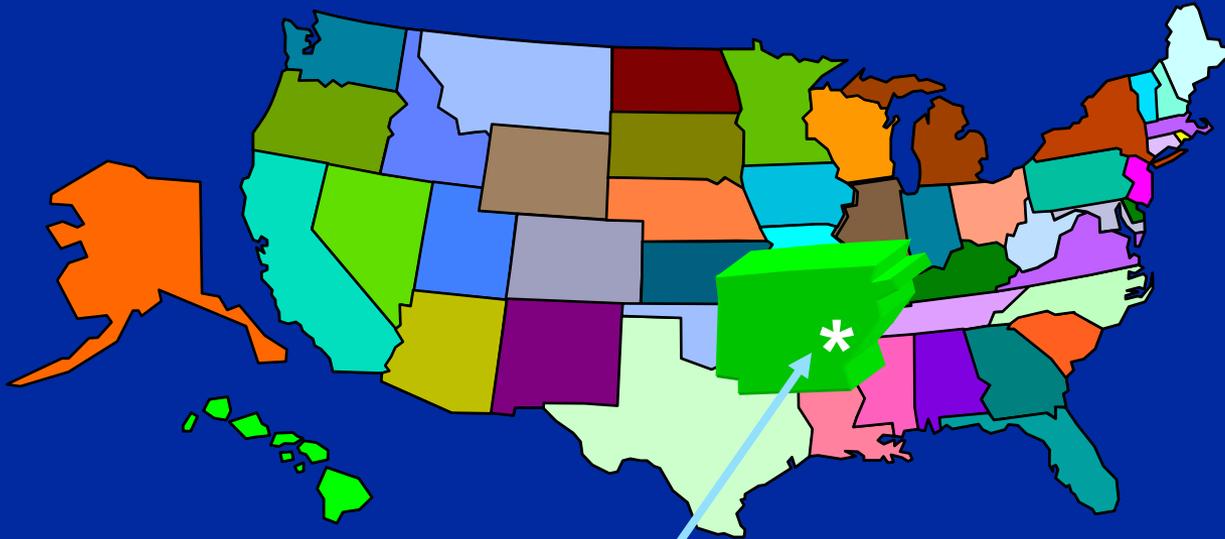
## **Chemistry (NCTR)**

Richard D. Beger, Ph.D.  
Rich Edmonson, Ph.D.  
J. Pat Freeman, Ph.D.  
Thomas M. Heinze  
Richard Jones, Ph.D.  
Jackson Lay, Ph.D.

David T. Gibson, Ph.D., Iowa  
E. Brian Russell, UAMS  
David E. Wennestrom, Ph.D., UAMS  
Gerben J. Zylstra, Ph.D., Rutgers

Sandra Malone

# Thank You



**National Center for  
Toxicological Research  
Jefferson, Arkansas, U.S.A.**

[ccerniglia@nctr.fda.gov](mailto:ccerniglia@nctr.fda.gov)